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Neurophysiologic and proteomic investigations of experience-dependent plasticity in the somatosensory cortex of the adult mouse following chronic whisker stimulation

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RESUME

Les follicules des vibrisses des rongeurs sont représentés sous la forme d'une carte topographique dans le cortex à tonneaux. Lorsque un groupe de vibrisses est coupé pendant plusieurs jours chez un rongeur adulte, en laissant les autres vibrisses intactes, le champ réceptif des neurones du cortex à tonneaux est modifié, ce qui démontre que les cartes corticales sont plastiques. Dans notre étude, une expérience sensorielle a été induite chez une souris adulte se comportant librement en stimulant chroniquement une de ses vibrisses pendant 24h.

Par une analyse des potentiels de champ locaux, nous démontrons que les caractéristiques spatiotemporelles du flux d'excitation évoqué par la vibrisse principale (VP) dans la colonne corticale correspondante à la vibrisse stimulée n'est pas altéré. Par contre, l'enregistrement des potentiels d'actions d'un total de 1041 neurones à travers le cortex à tonneaux révèlent plusieurs modifications de l'activité neuronale. L'activité spontanée ainsi que la réponse évoquée par la VP sont déprimées dans la colonne corticale stimulée (nombre moyen de potentiels d'action évoqués par la VP diminue de 25 % et 36% dans la couche IV et les couches II&III). La réponse des neurones à la vibrisse stimulée diminue également dans les colonnes corticales adjacentes, «non-stimulées». La dépression de l'activité spontanée et de la réponse à la VP est localisée à la colonne corticale stimulée. Dans le tonneau stimulé, la première partie de la réponse à la VP n'est pas affaiblie, démontrant que la dépression de la réponse n'est pas due à un phénomène de plasticité sous-corticale ou thalamocorticale. La stimulation chronique d'une vibrisse entraîne une augmentation du nombre de synapses GABAergiques dans la couche IV du tonneau correspondant (Knott et al, 2002). Dès lors, nos résultats suggèrent qu'une augmentation de l'inhibition dans le tonneau stimulé serait à l'origine de la diminution des potentiels d'action évoqués par la vibrisse stimulée et en conséquence de l'amplitude du flux d'excitation vers les couches II&III puis vers les colonnes corticales adjacentes. Toutes les réponses des neurones du tonneau stimulé ne sont pas déprimées. Les réponses des neurones à la vibrisse voisine caudale à VP diminuent dans la couche IV (42%) et dans les couches II&III (52%) mais pas les réponses aux 7 autres vibrisses voisines. Les entrées synaptiques en provenance de la vibrisse caudale pourraient avoir été spécifiquement déprimées en raison d'une décorrélation prolongée entre l'activité évoquée dans les chemins sensoriels relatifs à la vibrisse stimulée et à la vibrisse caudale, spécificité qui découlerait du fait que, parmi les vibrisses voisines à la VP, la vibrisse caudale génère les réponses les plus fortes dans la colonne corticale. Quatre jours après l'arrêt de la stimulation, l'activité neuronale n'est plus déprimée; au contraire, nous observons une potentiation des réponses à la VP dans la couche IV de la colonne corticale stimulée.

De plus, nous montrons que l'expression des protéines GLT-1 et GLAST, deux transporteurs astrocytaires du glutamate, est augmentée de ~2.5 fois dans la colonne corticale stimulée, indiquant l'existence d'une «plasticité gliale» et suggérant que les cellules gliales participent activement à l'adaptation du cerveau à l'expérience.

ABSTRACT

In the barrel cortex, mystacial whisker follicles are represented in the form of a topographic map. The selective removal of a set of whiskers while sparing others for several days in an adult rodent alters receptive field of barrel cortex neurons, demonstrating experience-dependent plasticity of cortical maps. Here sensory experience was altered by chronic stimulation of a whisker for a 24h period in a freely behaving adult mouse.

By means of an evoked local field potential analysis, we show that chronic stimulation does not alter the flow of excitation evoked by the principal whisker (PW) in the stimulated barrel column. However, the recording of neuronal firing from a total of 1041 single units throughout the barrel cortex reveals several changes in neuronal activity. Immediately after chronic stimulation, spontaneous activity as well as PW-responses are depressed in the stimulated barrel column (mean number of spikes per PW-deflection decreases by 25% and 36% in layer IV and layers II&III, respectively). Neuronal responses towards the chronically stimulated whisker are also significantly depressed in layers II&III of the adjacent "non-stimulated" barrel columns. The depression of both spontaneous activity and PW-responses are restricted to the stimulated barrel column. The earliest time epoch of the PW-response in the stimulated barrel is not depressed, demonstrating that the decrease of cortical responses is not due to subcortical or thalamocortical plasticity. The depression of PW-response in the stimulated barrel correlates with an increase in the number of GABAergic synapses in layer IV (Knott et al., 2002). Therefore, our results suggest that an increase in inhibition within the stimulated barrel may reduce its excitatory output and accordingly the flow of excitation towards layers II&III and the subsequent horizontal spread into adjacent barrel columns. Not all responses of neurons in the stimulated barrel are depressed. Neuronal responses towards the caudal in-row whisker decrease by 42% in layer IV and 52% in layers II&III but responses to the other 7 immediate surround whiskers (SWs) are not affected. The synaptic inputs from the SW that elicit the strongest responses in the stimulated barrel may have been specifically depressed following a prolonged period of diminished coherence between neuronal activity evoked in the pathways from the chronically stimulated whisker and from its surrounding in-row whisker. Four days after the cessation of the stimulation, depression of neuronal activity is no longer present; on the contrary, we observe a small but significant potentiation of PW-responses in layer IV of the stimulated barrel column.

Moreover we show that the expression of astrocytic glutamate transporters GLT-1 and GLAST proteins were both upregulated by ~2.5 fold in the stimulated barrel column, which indicates that glial cells exhibit experience-dependent functional changes and could actively take part in the adaptation of the cerebral cortex to experience.

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CONTENTS

List of abbreviations

A	ampere
A/m	ampere per meter of conductor
AMPA	amino-3-hydroxy-5-methyl-4-isoxiazole propionic acid
AMPA-R	amino-3-hydroxy-5-methyl-4-isoxiazole propionic acid receptor
AP	action potential
BDNF	brain derived neurotrophic factor
$b_{y.x}$	Regression coefficient
CRE	cyclic adenosine monophosphate responsive promoter element
CSD	current source-density
CTB	cholera toxin B subunit
d	days
D-APV	D-2-amino-5-phosphonovalerate
4d-after-STIM	group of mice that were stimulated 24h for their C2-whisker then returned 4 days to their home cage
2-DG	2-deoxyglucose
EAAT	excitatory amino acid transporter
epsp	excitatory postsynaptic potential
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
GLAST	rat glutamate transporter EAAT1
GLT-1	rat glutamate transporter EAAT2
GS	glutamine synthetase enzyme
h	hours
HRP	horse-radish peroxidase
ipsp	inhibitory postsynaptic potential
i.p.	intra-peritoneal
IQ	interquartile range (Q75-Q25)
LFP	local field potential
LH	latency histogram
µm	micrometer
ms	milliseconds
NMDA	N-methyl-D-aspartate
NMDA-R	N-methyl-D-aspartate-receptor
NON STIM	non stimulated group of mice
P.B.	phosphate buffer
POm	medial division of the posterior thalamic nucleus
PW	principal whisker
PMBSF	posteromedial barrel subfield
PrV	principal sensory nucleus
PSTH	peristimulus time histogram

R^2	Determination coefficient for regression best fit line
RM	response magnitude
rms	root mean square
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SmI	somatosensory primary area
SpVc	caudal division of the spinal sensory nucleus
SpVi	interpolaris division of the spinal sensory nucleus
SRF	surround receptive field
SW	surround whisker
24h STIM	Twenty-four hour C2-whisker stimulated mice
V	volt
VB	Ventro-basal thalamic nuclei
VPM	Ventro-postero medial thalamic nuclei
VSD	Voltage sensitive dye

Figures and Tables

INTRODUCTION

Sensory experience shapes functional neuronal circuits in the developing and adult cerebral cortex. Throughout the past two decades, it has become increasingly evident that modifications in the use of sensory receptors and associative learning can induce functional changes in the neuronal circuits of the adult mammalian cerebral cortex, characterized as "cortical plasticity", that consequently alter the way it processes neuronal information (Kaas et al., 1983; Merzenich et al., 1984; Pons et al., 1991; Weinberger, 1993; Armstrong-James et al., 1994; Wang et al., 1995; for a review: Buonomano and Merzenich, 1998; Foeller and Feldman, 2004). The understanding of cortical plasticity mechanisms constitutes one of the most captivating research field in science because it should give us a glimpse on how we remember past events, how we learn and adapt our behaviour and how our perception of the world continuously evolves throughout life.

It is widely admitted that activity-dependent functional changes in neuronal circuits rely on synaptic plasticity, i.e. sustained modifications in neuronal connectivity in response to different patterns of neuronal activity. Synaptic plasticity can occur at different levels 1) regulations of synaptic strength, such as the long term potentiation (LTP) and depression (LTD) of excitatory postsynaptic potentials demonstrated *in vitro* (Bliss and Lomo, 1973; Lynch et al., 1977; Martin et al., 2000), 2) structural rearrangement of the neuronal network such as axonal sprouting (Darian-Smith and Gilbert, 1994), 3) synaptogenesis and synaptic elimination (Chang and Greenough, 1982; Knott et al., 2002). Remarkably, the notion of synaptic plasticity itself, its potential functions as well as the fundamental principles that drive it into the neuronal networks were summarized in a simple and intuitive postulate formulated more than 50 years ago by Donald Hebb (1949) in one of the pioneering books in neuroscience "The organization of behaviour": "*When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic changes takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased*". This basic model also predicted that the lack of correlation between activity in the two connected neurons leads to a decreased efficiency of their connections. The concept of the "Hebbian synapse" had and still has a profound influence in neurosciences and on neuroscientist's brains. Today, this postulate is often rephrased in the sense that synaptic plasticity is driven by correlations in the firing activity of pre- and postsynaptic neurons. Experimental and modelling studies have strengthened and developed the Hebbian theory of synaptic plasticity, and moreover

have revealed that plasticity also occur at inhibitory synapses (Cruikshank and Weinberger, 1996; Buonomano and Merzenich, 1998; Gaiarsa et al., 2002).

Given the extreme complexity of the brain synaptic network and of the synaptic properties, it remains technically and conceptually difficult to establish a link *in vivo* between synaptic plasticity events, the functional modifications in brain networks and the consequences onto information processing. However, several lines of evidences indicate that synaptic plasticity is the fundamental cellular property underlying both the final establishment and the refinement of neuronal circuits during neonatal development and their modifications in function of experience in adults (Buonomano and Merzenich, 1998; Foeller and Feldman, 2004). A major hypothesis in neuroscience states that synaptic plasticity allows the brain to sustain learning and memory (Hebb, 1949; Martin et al., 2000).

The barrel cortex of rodents as a tool to study cortical plasticity. Experience-dependent plasticity of cortical maps shares common properties in all mammals, including the human species (Calford, 2002; Chen et al., 2002). The receptive field maps in the rodent somatosensory cortex have been extensively used as a model to gain knowledge about the mechanisms of experience-dependent plasticity in the brain. Particularly, the representations of the facial whiskers in the primary somatosensory cortex (SmI) proved major advantages. Not only the whiskers are readily accessible for controlled manipulations of sensory experience but the fairly small clusters of neurons dedicated to individual whisker follicles are easily identifiable histologically and functionally throughout the central nervous system (Woolsey and Van Der Loos, 1970; Van Der Loos, 1976; Belford and Killackey, 1979), which allows one to track the potential effects produced by changes in the periphery at the precise locations of the corresponding brain areas. To understand the expression of plasticity, the knowledge of neuronal pathways is crucial. Thus, in the following sections, we will outline the anatomical and functional basic characteristics of the whisker sensory system as well as some of the principles of experience-dependent plasticity in this system.

I. The somatosensory cortex

I.1. The cerebral cortex

The entire telencephalon is covered by a continuous laminated neuronal structure termed the cerebral cortex. The cell bodies of cortical neurons are arranged in cytoarchitecturally distinct layers parallels to the brain surface (White, 1989 for a review). The cerebral cortex may be defined as the higher order centre of the brain. It is composed of embryological divisions called lobes which are organized and localized with respect to primary attributes, such as motor, sensory, and emotional senses, though functionally interdependent (Kandel et al., 2000 for a review). These regions are intimately connected with high order subcortical regions as well as with the brainstem and the spinal cord. Phylogenetically, the cerebral cortex of all vertebrates

comprises a paleocortex, which includes olfactory cortex and cortical regions in the temporal lobe, and an archicortex, which is centred on the hippocampus (Nieuwenhuys et al., 1981 for a review). The mammalian brain moreover possesses a third type of cortex typically comprising six cellular layers, the neocortex. The neocortex is the biggest cortical structure in mammals and is particularly developed in primates. It receives all its subcortical afferent inputs from the thalamus. Although all areas of the neocortex have six layers, the relative thickness and the distribution of the various cell types within these layers vary in function of the cortical regions, which have formed the basis for dividing the cortex into cytoarchitectonic areas (Brodmann, 1909). The cortical areas are functionally differentiated (Kandel et al., 2000 for a review).

I.2. The primary somatosensory cortex

In all mammals, the sensory periphery projects onto particular areas of the neocortex via nerve fibres and synaptic subcortical relays in such a way that topographic maps are set in the auditory, visual and somatosensory cortices (Kandel et al., 2000). The primary somatosensory cortex (SmI) is located in the parietal cortex and is devoted to the sensitivity of the body. SmI is a primary area because it is the first cortical region to receive ascending sensory signals from the periphery. It therefore contains a "first-order" representation of the body surface. The SmI cortex provides the substrate for conscious perceptions of somatic sensations and also plays a role in motor control via reciprocal projections with motor cortex (in rodents: Izraeli and Porter, 1995). A prominent principle in the organization of the mammalian sensory pathways is the topologically-ordered relationship between the sensory periphery and its representations in the brain. Central representations can be described as maps that are homeomorphic with respect to the distribution of the sensory receptors in the corresponding peripheral regions of the body. The systematic correspondence of peripheral receptors in body regions with specific functional areas of the SmI is termed somatotopy.

II. Model used in this study: the whisker sensory system of rodents

II.1. The "barrel cortex".

In rodents, a large representation cortical field is dedicated to the facial tactile whiskers, occupying up to one third of the total surface of their SmI (Woolsey and Van Der Loos, 1970). The relative importance of the SmI area dedicated to whiskers as compared to the actual surface of the body they covered illustrates a typical characteristic of cortical map: the size of a cortical representation of a body region is proportional to its peripheral innervation density (Lee and Woolsey, 1975). Environmental clues retrieved through their tactile whiskers are crucial for rodents notably when visual exploration is prevented, which is often the case for these mostly

nocturnal animals. Rodents extensively use their whiskers for behavioural functions ranging from the exploration of their gross environment (Vincent, 1912) to the judgment of subtle surface textures (Carvell and Simons, 1990).

Whisker follicles map in a one-to-one somatotopic manner onto clusters of neurons in layer IV of the contralateral SmI that were termed barrels because of their three-dimensional structure (Woolsey and Van Der Loos, 1970, Welker, 1976). As seen in layer IV sections made tangentially to the brain surface and stained for Nissl-substance, barrels are oval-shaped ensembles of granular neurons. Each layer IV barrel is part of one functional cortical column, i.e. distinct functional modules that extend vertically throughout the thickness of the cortex (Mountcastle, 1957), that were therefore termed "barrel column". The border of a barrel ("walls") is enriched in cell bodies as compared to its centre hollow. Surrounding the barrels are cell-poor regions called septa. The spatial arrangement of these multi-cellular cytoarchitectonic entities reproduces topographically the organization of the whiskers onto the muzzle of the animal. The area of SmI containing the representation of the whiskers is referred as the "barrel cortex" (figure 1). The part of the barrel cortex devoted to the largest whiskers stereotypically arranged in five rows on the whiskerpad defined the posteromedial barrel subfield region (PMBSF).

A wealth of anatomical and physiological experiments has demonstrated that each barrel primarily processes the sensory signals ascending from the anatomically corresponding whisker onto the whiskerpad (Simons, 1978; Simons and Carvell, 1989; Armstrong-James and Fox, 1987; for a review: Moore et al., 1999; Miller et al., 2001). Among these studies, one is of particular interest here because it demonstrates a basic concept that govern the sensory cortical map mentioned above: the size of a particular barrel is proportional to the number of myelinated sensory nerve fibers innervating the corresponding whisker follicle (Welker and Van Der Loos, 1986).

II.2. Anatomical overview of the whisker-to-barrel sensory pathway

Motile tactile sensory organs on the whiskerpad of rodents

Whiskers, or vibrissae, are specialized hairs found on the muzzle of many mammalian species in a region called "whiskerpad". Each whisker, itself consisting of inert hair material, emerges from a follicle containing mechanoreceptors sensitive to movement of the hair fiber. In mice, these tactile sensory organs are arranged in five rows of large whiskers (mystacial vibrissae; figure 1) bordered caudally by four straddlers. Near the snout are also found numerous smaller whiskers, less densely innervated than the mystacial whisker follicles, the rostral vibrissae. Each follicle is innervated by a sensory follicular nerve. Follicular nerves get together into row nerves which merge caudally into the infraorbital nerve, a branch of the trigeminal nerve (Dorfl, 1985). Trigeminal mechanosensory neurons emerging from each follicle are sensitive exclusively to the movement of the associated whisker (Gibson and Welker, 1983).

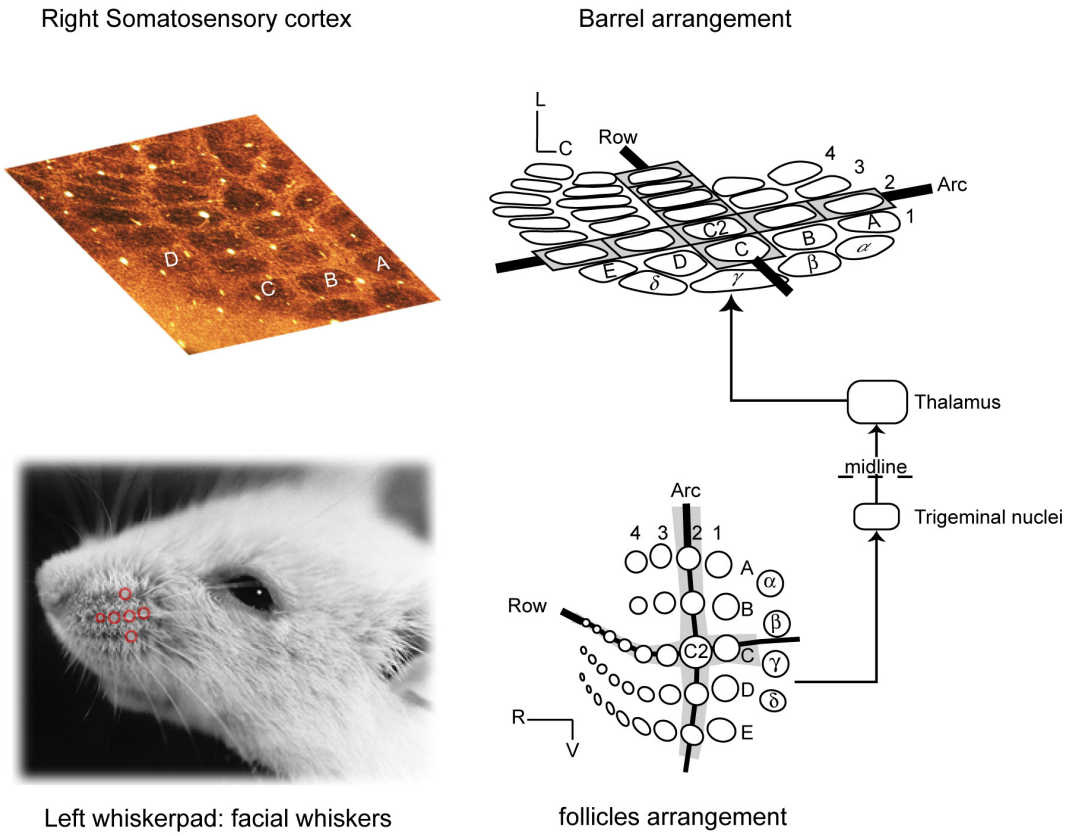


Figure 1. Schematic illustration of the sensory pathway from the whisker follicles to the barrel columns of the somatosensory primary cortex (SmI).

Photographs and drawings illustrating the arrangement of the whisker follicles onto the left whisker pad and of the layer IV barrels in the contralateral somatosensory cortex of a mouse from the NOR strain. In the above micro-photograph, the topographic ordering of barrels is revealed by a cytochrome oxydase staining of a tangential section trough layer IV.
Facial whisker follicles are arranged in five caudorostral rows (A-E). Within each row, follicles are numbered in the caudorostral direction so that whiskers from the different rows labelled with the same number form vertically oriented arcs of whiskers. Four additional caudal whiskers follicles straddle the rows and are labelled with Greek symbols. Red circles on the photographs indicate row C and Arc-2 whiskers. In the lemniscal pathway, the sensory inputs from each follicle converge to the corresponding layer IV barrel hollows of the contralateral SmI, with synaptic relays in the trigeminal nuclei of the brainstem and in the ventro-basal nuclei of the thalamus. As a result of this strict somatotopy, barrels are arranged in rows and arcs and are named according to the whisker that project to them, their principal whisker (PW). Orientation bars: R= rostral, V= ventral / C= caudal, L= lateral.

Finally, follicles of mystacial whiskers are moved by specific striated muscles, the follicular muscles, innervated by a branch of the facial nerve (Dorfl, 1982). Follicular muscles protract the whiskers in the caudorostral direction. These muscles allow the typical “whisking” behaviour of rodents: while exploring their environment, rodents engage their whiskers in rhythmic movement at frequencies between 5-15 Hz (Welker, 1964; Carvell and Simons, 1990).

Ascending pathways from whisker follicles to cortex

Along the ascending sensory pathway from the whisker follicles to the contralateral SmI, homeomorphic whisker representations that can be visually identified with basic histological staining techniques are present at each synaptic station The central axons of trigeminal neurons connected to individual whisker follicles converge onto discrete cytoarchitectonic domains called “barrelettes” in three out four of the nuclei of the trigeminal sensory complex: principal sensory nucleus (PrV), interpolaris (SpVi) and caudal division of the spinal sensory nucleus (SpVc) (Belford and Killackey, 1979; Ma, 1991; Welker et al., 1996). Projections from trigeminal nuclei terminate in the ventrobasal nucleus (VB in mice; ventral posterior medial or VPM in rats), the thalamic lemniscal relay for somatic sensations, and in the medial division of the posterior nucleus (POm) of the contralateral thalamus. The arrangement of neurons of the VB nucleus in drop-shaped clusters termed “barreloids” mirrors that of the large whiskers on the whiskerpad (Van Der Loos, 1976). Thalamocortical (TC) neurons originating from VB then convey the fastest representation of the stimulus to the SmI cortex where they arborize and make excitatory synapses (Keller et al., 1985; Agmon and Connors, 1991; Agmon and Connors, 1992). Using injections of anterograde tracers in VB or anterograde degeneration methods, it was then established that in the mouse somatosensory system, TC terminals were concentrated in layer IV, lower layer III and to some extent around the border between layer V and VI (Killackey, 1973; White, 1978; Caviness and Frost, 1980; Frost and Caviness, 1980; White et al., 1985; Bernardo and Woolsey, 1987). TC-axon terminals originating from individual barreloids are strongly clustered and project mainly in the corresponding barrels at the level of layer IV in both rats and mice (Killackey, 1973; White, 1978; Jensen and Killackey, 1987; Land et al., 1995; Keller and Carlson, 1999; Arnold et al., 2001; Gheorghita-Baechler and Welker, 2005). Cell-sparse inter-barrel region, called septa, received TC-inputs from POm (Koralek et al., 1988; Chmielowska et al., 1989; Lu and Lin, 1993). These latter projections are characterized by a less precise somatotopy, being part of a non-lemniscal pathway.

II.3. Processing of sensory signals in the barrel cortex

In agreement with the anatomical organization of the whisker-to-barrel projections, electrophysiological measurements of neuronal activity across the barrel cortex of anaesthetized rodents have shown that the fastest neuronal responses evoked by the deflection of a whisker occur in layer IV neurons of the anatomically corresponding barrel (Simons, 1978; Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Welker et al., 1993; Moore and Nelson, 1998; Petersen and Diamond, 2000; Zhu and Connors, 1999; Brecht and Sakmann, 2002a). Layer IV therefore constitutes the first stage in cortical processing of sensory signals.

Intracolumnar flow of excitation

Following their activation by TC-inputs, layer IV neurons subsequently relay the evoked sensory signal to layers II&III neurons and this transmission is at least initially strongly confined to the region situated above the home barrel within the barrel column (Armstrong-James et al., 1992; Welker et al., 1993; Laaris et al., 2000; Petersen and Sakmann, 2001; Feldmeyer et al., 2002; Petersen et al., 2003a). The anatomical substrates for the columnar propagation of excitation are the vertical axonal projections of layer IV neurons connecting layers II&III pyramidal neurons. Those projections are mainly confined to the cross sectional area of their home barrel, defining an anatomical barrel column (Simons and Woolsey, 1984; Feldmeyer et al., 2002; Lubke et al., 2003). Layers II&III pyramidal neurons send axonal projections vertically to infragranular layers and horizontally into adjacent barrel columns (Gottlieb and Keller, 1997; Brecht et al., 2003; Petersen et al., 2003a). The activation of infragranular layers V and VI following the deflection of a whisker however results from several pathways. Layer V neurons are strongly connected by layers II&III neurons (Harris and Woolsey, 1983; Bernardo et al., 1990b; Gottlieb and Keller, 1997; Zhang and Deschenes, 1998; Schubert et al., 2001). In addition, they receive direct inputs from the lemniscal pathway on their soma and on their apical dendrites that ascend through layer IV, as well as from the non-lemniscal pathway (Koralek et al., 1988; Keller and White, 1989; Lu and Lin, 1993; Manns et al., 2004). A fraction of layer Vb neurons respond at similar latencies than layer IV neurons within the home barrel column upon the deflection of the corresponding whisker (Armstrong-James et al., 1992). The main inputs of layer VI are layer V collaterals, however they also receive some intracortical inputs from layers IV and II&III and some TC-inputs (Zhang and Deschenes, 1998; Staiger et al., 2000). While layer V and layer VI act as outputs of the cortex towards other cortical and subcortical areas (Welker et al., 1988; Deschenes et al., 1994), they also send numerous axon collaterals towards layer IV of the same and adjacent columns (Staiger et al., 1996b; Gottlieb and Keller, 1997; Zhang and Deschenes, 1997). Thus, infragranular layers may also play a role during whisker inputs processing within the barrel cortex (Wirth and Luscher, 2004).

Altogether, the principal flow of information through the cortical layers could be described as a "VB→layer IV→layers II&III→layer V/VI" pathway that initially restricts processing to one barrel column. However many other interlaminar and thalamocortical connections are present, including a robust non-lemniscal projections from POm to septal neurons (Hoeflinger et al., 1995; Kim and Ebner, 1999).

Intercolumnar flow of excitation

The flow of excitation evoked by TC-inputs in one barrel column invades adjacent barrel columns within 2-3 ms following the activation of layers II&III neurons by their layer IV counterparts (Armstrong-James et al., 1992; Kleinfeld and Delaney, 1996; Petersen and Sakmann, 2001; Petersen et al., 2003a). As a consequence, cortical

neurons not only respond to the anatomically corresponding whisker, the principal whisker (PW), but also to surrounding whiskers (SWs) (Welker, 1976; Simons, 1978; Armstrong-James and Fox, 1987; Welker et al., 1993; Moore and Nelson, 1998; Zhu and Connors, 1999). In all cortical layers of a barrel column, neurons respond faster and stronger in response to deflections of the PW than to SWs, creating a spatiotemporal orderly whisker map across the barrel cortex. The set of whiskers on the whiskerpad that, when stimulated, affects the firing of a neuron is defined as its receptive field. Yielding the fastest responses, the PW determine the centre receptive field (CRF) of neurons. The surround receptive field (SRF) of barrel neurons generally comprise several whiskers adjacent to the PW and is thought to be generated for a large part intracortically through horizontal transcolumnar connections (Armstrong-James et al., 1991; Armstrong-James et al., 1992; Fox, 1994; Fox et al., 2003). Rodents usually engage concomitantly all their whiskers in the exploration of the environment; the intracolumnar connections in the barrel cortex allows the integration of information related to the movement of individual whiskers. This notably allows the animal to compare individual whisker informations, which is crucial for an appropriate representation of locations and shapes of surrounding objects (Mirabella et al., 2001).

Pathways for the building of cortical surround receptive fields

In layers II&III, the transcolumnar axonal projections of pyramidal neurons span several barrel columns and therefore are responsible for the relay of excitation evoked by their PW in surrounding columns (Gottlieb and Keller, 1997; Keller and Carlson, 1999; Brecht et al., 2003; Petersen et al., 2003a). These projections show a preferred orientation along the rows of barrel columns; using an *in vivo* voltage sensitive dye (VSD) imaging technique, it has been shown that the flow of excitation generated in supragranular layers by the deflection of a single whisker propagates preferentially along the same row axis (Petersen et al., 2003a). Such a transcolumnar pathway could also explain the tendency of in-row SWs to elicit stronger responses than other SWs in layers II&III neurons at both the subthreshold and suprathreshold levels (Chapin, 1986; Armstrong-James et al., 1992; Welker et al., 1993; Brecht et al., 2003; Petersen et al., 2003a). Similarly, signal processing in layer V is not columnar because the axonal collaterals from layers II&III to layer V as well as the axonal and dendritic arborizations of layer V neurons do not respect barrel column borders (Gottlieb and Keller, 1997; Petersen and Sakmann, 2001).

In both rats and in mice, layer IV neurons also respond to several SWs, at similar latencies than layers II&III neurons (Armstrong-James et al., 1992; Welker et al., 1993). The long latencies of SW responses in layer IV barrel suggest that they are not generated monosynaptically by TC terminals but rather rely on intracortical transcolumnar transmission (Armstrong-James et al., 1992). A transcolumnar route ending in layer IV barrels coming from infragranular layers have been described in a wealth of studies (Bernardo et al., 1990b; Chagnac-Amitai et al., 1990; Staiger et al., 1996b; Gottlieb and Keller, 1997; Zhang and Deschenes, 1997), however direct

connections between barrels in layer IV (Lubke et al., 2000; Petersen and Sakmann, 2000; Brecht and Sakmann, 2002a; Schubert et al., 2003; Staiger et al., 2004) or transcolumnar projections from supragranular layers (Lubke et al., 2000; Petersen et al., 2003a; Brecht et al., 2003) towards layer IV are present but sparse. The hypothesis that SW-responses depend on transcolumnar propagation of whisker-evoked sensory signals is still controversial (Armstrong-James et al., 1991; Goldreich et al., 1999; Fox et al., 2003). SW responses may have a subcortical origin: the SRF of layer IV neurons could be due to a divergence of TC-afferents. Using an electrode array, Petersen and Diamond (2000) have observed that the deflection of a single whisker can activate neurons in 1-7 barrel columns within 10 ms. However neurons in barrels adjacent to the barrel corresponding to that whisker usually respond at latencies > 10 ms. Thus, responses at short latencies could be explained by the observation that some TC projections extend beyond barrel borders in rats (Arnold et al., 2001) and in mice (Gheorghita-Baechler and Welker, 2005) while most of neuronal responses rely on transcolumnar propagation of excitation. It should be further noticed that despite the robust segregation of whisker sensory pathways, neurons in the subcortical synaptic relays also exhibit SRF (Ito, 1988; Armstrong-James and Callahan, 1991; Diamond et al., 1992; Brecht and Sakmann, 2002b). These observations have lead to the hypothesis that SW responses in barrel neurons could be driven directly by their TC-inputs. However it was claimed that thalamic neurons in VPM does not transmit their SRF to the barrel neurons (Armstrong-James et al., 1991; Armstrong-James and Callahan, 1991). This point will be further developed in the discussion.

The flow of inhibition within the barrel cortex

Excitatory and inhibitory neurons in layer IV are both excited monosynaptically by TC terminals and are reciprocally connected (White, 1978; Porter et al., 2001). Feedforward and feedback γ-aminobutyric acid (GABA)-inhibition within barrels should serve to enhance temporal resolution of the barrel network and to shape receptive fields (Welker et al., 1993; Pinto et al., 2000; Swadlow and Gusev, 2000). In correspondence with that, application of $GABA_A$ receptor antagonists *in vivo* results in an expansion of the SRF size of layer IV barrel neurons (Kyriazi et al., 1996b). Layer IV interneurons also project to supragranular layers, suggesting that the flow of inhibition after TC-input follows the flow of excitation along its intracortical course (Porter et al., 2001). It was observed *in vitro* in thalamocortical slices that the stimulation of a single barreloid evoked excitatory-VSD signal that was mainly restricted to a single barrel, the excitation spread in supragranular and infragranular layers of the corresponding and adjacent barrel columns only after $GABA_A$ receptor complete blockade with high concentration of biccuculine (Laaris et al., 2000). Thus, inhibition also limits both temporally and spatially the spread of excitation evoked in the barrel cortex upon whisker deflection. Finally, layer IV interneurons could mediate surround inhibition (Simons, 1985; Welker et al., 1993). In correspondence with that,

Porter et al. (2001) also showed that some interneurons in layer IV barrel send their projections in neighbouring barrels.

III. Experience-dependent plasticity in the somatosensory cortex

Receptive fields of neurons in the cerebral cortex are not rigid but on the contrary, they can be modified during the entire life by experience. Receptive field plasticity has been demonstrated in the cerebral cortex following several types of manipulations of sensory inputs: limb amputation or peripheral nerve damage (Merzenich et al., 1984; Merzenich et al., 1983), changes in visual and somatosensory experiences (Wiesel and Hubel, 1963b; Simons and Land, 1987; Diamond et al., 1993) or behavioural training (Recanzone et al., 1992; Siucinska and Kossut, 1996). It is widely accepted that experience-dependent changes in neuronal responses in the cerebral cortex are mediated by modifications at the synaptic level and that plasticity of the synaptic networks allows the brain to sustain learning related tasks and memory.

During the last decades, huge progresses have been made towards a comprehension of the mechanisms governing experience-dependent plasticity in the cerebral cortex. A significant part of the advances realized in this field comes from studies on the barrel cortex of rodents. The whisker receptive field of the barrel cortex exhibits activity-dependent plasticity upon several kinds of alterations in the pattern of whisker experience in both the neonatal immature and the mature somatosensory cortex (Buonomano and Merzenich, 1998).

The impact of altered experience upon neuronal circuits changes dramatically as a function of age. In general, at early postnatal ages, TC-synapses are predominant sites for experience-dependent plasticity in the barrel cortex and layer IV receptive fields are highly sensitive to sensory experience (Simons and Land, 1987; Fox, 1992). Within the two first postnatal weeks, receptive fields of neurons in layers II&III become more sensitive to modifications in sensory experience than in layer IV (Fox, 1992; Glazewski et al., 1996), suggesting that the primary sites of plasticity shift from the thalamocortical to the intracortical synaptic network as the brain matures. In the adult, alterations in the use of whiskers can still affect functional receptive fields through mechanisms that operate at intracortical synapses, mainly at layer IV to layers II&III synapses as well as at inter-barrel column synaptic connections (Diamond et al., 1993; Armstrong-James et al., 1994; Glazewski et al., 1996; for a review: Buonomano and Merzenich, 1998; Fox, 2002; Foeller and Feldman, 2004). However, some capacities for thalamocortical experience-dependent plasticity remain throughout adulthood (Armstrong-James et al., 1994; Wallace and Fox, 1999).

A wealth of *in vitro* and *in vivo* studies in the barrel cortex of rodents have shown that activity-dependent plasticity of neuronal networks is more prominent during the two first weeks of life (for a review: Fox, 1992; Feldman et al., 1999; Foeller and Feldman,

2004). Thus, a great part of our knowledge about the mechanisms of synaptic plasticity in the cortex comes from experiments conducted during neonatal development. For this reason, although the present study is dedicated to plasticity in the adult, the main forms of plasticity observed during neonatal development will also be described in the following sections.

III.1. Developmental plasticity in the somatosensory system

Neuronal activity drives the neonatal development of the barrel cortex. In mammals, whereas the gross anatomy of the central nervous system is established at birth, neuronal circuits are still largely immature (Katz and Crowley, 2002). The lack of appropriate sensory experience during the early days of life strongly perturbs the maturation of neuronal networks at the subcortical and cortical levels (Wiesel and Hubel, 1963a; Wiesel and Hubel, 1963b; Van Der Loos and Woolsey, 1973; Belford and Killackey, 1980). In the barrel cortex of rodents, the sequence of events that will lead to the refinement of first thalamocortical then intracortical wiring during the first two weeks of life, resulting in the precise morphological and functional somatotopy between whiskers and barrel columns, crucially depends on the activity evoked at the peripheral follicular receptors (Van Der Loos and Woolsey, 1973; Simons and Land, 1987; Fox, 1992; Stern et al., 2001; Rema et al., 2003; Hage et al, 2003). It is thought that the activity generated in the whisker-to-barrel pathway drives competitive interactions between synaptic inputs within the neuronal network and consequently shapes these with high specificity through synaptic plasticity mechanisms.

Lesion-induced thalamocortical plasticity: follicular or trigeminal nerve lesions

In mice, TC-afferents already exhibit a correct laminar positioning and a topological precision at P1 however their axonal arbors are more diffusely distributed than in adults and barrel patterns are not visible (Agmon et al., 1995; Hage, 2003). The typical clustering of cortical neurons in individual barrel structures and the segregation of TC-axonal arbors in those barrel domains are clearly defined at P4 (Rice and Van Der Loos, 1977; Erzurumlu et al., 1990; Hage, 2003). In both rats and mice, the destruction of whisker follicles or the sectioning of the infraorbital branch of the trigeminal nerve between P0 and P6 causes abnormal segragation of TC-terminals in adults, no longer restricted to barrel domain (Jensen and Killackey, 1987), and disrupts normal development of cytoarchitectonic barrel structures (Van Der Loos and Woolsey, 1973; Killackey et al., 1976; Harris and Woolsey, 1981; Schlaggar et al., 1993). Maximal perturbations occur when follicles are lesioned at P0 while after P6, TC-axonal arborizations and barrel patterning are no more sensitive to damage at the periphery, which delimit a critical period for large scale morphological plasticity (Rice and Van Der Loos, 1977; Jeanmonod et al., 1981; Mccasland et al., 1992). Chronic perfusion of the somatosensory cortex with the competitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist D-2-amino-5-phosphonovalerate (D-APV) partially prevents the rearrangements of the somatotopic patterns of TC-axons induced by

selective follicular destruction in neonates (Schlaggar et al., 1993), which indicates that postsynaptic activity plays a prominent role in structural plasticity during the critical period. However, substantial reorganization persists under activity blockade (Chiaia et al, 1994a,b), suggesting that structural plasticity has an activity-dependent component, that is driven by a reduction in sensory-evoked activity of the lesioned afferents, and an activity-independent component that may be driven by the loss of access to a peripheral trophic factor (Calia et al, 1998).

Experience-dependent thalamocortical plasticity: innocuous alterations in sensory experience

Unlike peripheral lesions, the selective removal of whiskers, through either clipping or trimming, leaves the sensory pathways intact. Removal of all or a subset of whiskers while sparing the others from P0 does not perturb the development of a normal pattern of TC-afferents and barrels (Fox, 1992; Keller and Carlson, 1999). However, it induces long-lasting modifications of the functional receptive fields in layer IV that can last throughout adulthood (Simons and Land, 1987; Fox, 1992; Rema et al., 2003). For instance, following univibrissa rearing since birth in rats, a procedure consisting in depriving all but one whisker, neurons in the barrels corresponding to deprived whiskers acquire enhanced responses to the spared whisker as measured in 1-3 months old animals (Fox, 1992). The short latency component (<10 ms poststimulus) of whisker-evoked responses are often used to evaluate whether experience-dependent changes have a subcortical origin because spikes evoked at short latency are related mainly to the TC-volley (Armstrong-James and Fox, 1987; Armstrong-James and Callahan, 1991; Fox, 1992; Armstrong-James et al., 1994). Since short latency responses are increased in deprived layer IV neurons upon the deflection of the spared whisker, receptive field plasticity should reflect fine scale alterations in the distribution of TC-synapses (Fox, 1992), not detected by histological techniques that reveal somatotopic patterns. Thus, it is thought that neuronal activity regulates fine-scale but not large-scale topography during the early postnatal period.

Mechanisms of TC-plasticity during the early postnatal period

One classic hypothesis is that layer IV receptive fields are refined following typical Hebbian-like modulations of synaptic circuits during the early postnatal period (Hebb, 1949; Stent, 1973; Bienenstock et al., 1982; Cruikshank and Weinberger, 1996). It is thought that spontaneous activity and evoked activity generated in the subcortical pathways and synaptic plasticity mechanisms drive the refinement of pre-established immature TC-projections that form the typical highly patterned topography seen in adult cerebral cortex by retraction of aberrant branches and addition of branches inside barrels (Levay et al., 1978; Levay et al., 1980; Agmon et al., 1995; Hage, 2003). Based on the pre-established TC-afferents topography, inputs from a specific whisker should be reinforced and fixed at areas of maximal convergence because of adequate correlations of pre- and post-synaptic activity. It should be weakened and subsequently

eliminated where inputs from another particular whisker dominates, leading to a precise somatotopic patterning of TC-projections as seen in adults. Such processes could be hypothesized to explain the expansion of the spared whiskers representations following selected whisker deprivation in neonates: sparing a subset of whisker may results in alterations of TC-interactions leading to the maintenance of spared inputs in deprived area of the cortex, as it was proposed in the visual cortex following monocular deprivation (Antonini and Stryker, 1996).

LTP and LTD at TC-synapses during the early postnatal development. Experience-dependent plasticity of layer IV receptive fields can still be induced in mature rats using whisker pairing (Diamond et al, 1993; Armstrong-James et al, 1994) or chessboard deprivation (Wallace and Fox, 1999). However, layer IV receptive fields' plasticity is maximal during the first postnatal week (Foeller and Feldman, 2004 for a review). For instance, increased responsiveness to the single spared whisker in layer IV neurons of deprived barrels is most powerful when plucking begins at P0 and declines to almost undetectable level by about P4 (Fox, 1992). This period is coincident, although smaller, with the period during which TC-synapses exhibit LTP and LTD. Indeed, LTP and LTD are inducible i*n vitro* only before P8 by means of TC-afferents stimulation paired with depolarization of the postsynaptic neurons to 0-10 mV and –50 mV respectively (Crair and Malenka, 1995; Feldman et al., 1998). Therefore, LTP and LTD at TC-synapses have been proposed as candidate mechanisms of experience-dependent plasticity during the early postnatal development.

Other correlative evidences reinforce this hypothesis: genetic and pharmacological manipulations that block LTP and LTD *in vitro* also disrupt the development of TC-circuits *in vivo* (Feldman et al., 1999). Finally, LTP at TC-synapses is dependent on the activation of NMDA receptors (NMDA-Rs) (Crair and Malenka, 1995). NMDA-Rs appear to be involved in unmasking amino-3-hydroxy-5-methyl-4-isoxiazole propionic acid (AMPA) currents, perhaps by the insertion or the modifications of AMPA receptors (AMPA-Rs) channels to synapses (Feldman et al, 1999). The decrease in the duration and the relative amplitude of NMDA-R currents as compared to AMPA-R currents around P8 has been suggested to explain the weakening of layer IV plasticity both *in vivo* and *in vitro* (Feldman et al., 1999). It was also proposed that as the inhibitory system matures, the increased inhibition of dendrites enhances the threshold for NMDA-R dependent plasticity (Agmon and Odowd, 1992). Thus, as proposed in the visual cortex (Katz and Shatz, 1996), TC-remodelling during development could be modulated by LTP and LTD. LTP induced at a subset of TC-synapses would lead to the stabilization of axonal and dendritic branches bearing those synapses while LTD would lead to the retraction of dendritic and axonal branches.

Developmental plasticity and the GABAergic system. Spontaneous and evoked activity were shown to be necessary for the development of inhibitory circuits in various brain regions (Gaiarsa et al., 2002). In rats, whisker deprivation starting from the first

postnatal week leads to a drastic two thirds reduction of the number of GABAergic synapses normally contacting dendritic spines in the layer IV of the deprived barrel columns that is not due to a reduction of the number of dendritic spines (Micheva and Beaulieu, 1995). GABA-mediated inhibition is known to shape receptive field properties (Welker et al., 1993; Kyriazi et al., 1996b; Pinto et al., 2000; Swadlow and Gusev, 2000). Therefore, the decrease in intracortical GABAergic innervation following sensory deprivation in neonates correlates well with the broadening of receptive fields observed following whisker deprivation started from P0 (Simons and Land, 1987). A decrease inhibition in deprived cortex could also be hypothesized to participate to the expansion of spared whisker representation (Fox, 1992), by decreasing the vetoing of TC-inputs.

Experience-dependent plasticity at cortico-cortical synaptic connections

Univibrissa rearing or all whisker deprivation from the beginning of the second week of the postnatal period profoundly alter layers II&III receptive fields. Deprivation of all whiskers during the second postnatal week leads to a broadening of receptive field in layer II&III (Stern et al., 2001). Univibrissa rearing beginning at P6 induces potentiation of responses to the spared whisker and depression of responses to the PW in layers II&III but not in layer IV of the deprived barrel columns adjacent to the barrel column corresponding to the spared whisker (Fox, 1992). Given that layer IV neuronal responses are unaltered, experience-dependent changes in neuronal responses should rely almost entirely in plasticity at cortical circuitry communicating between layer IV and layers II&III or within layers II&III circuitry.

In order to interpretate receptive field plasticity induced during the second postnatal week, it is important to note that this period coincides with the emergence of receptive fields in layers II&III neurons and that normal sensory experience is crucial for their establishment. While layer IV subthreshold receptive field are already mature at P12 as measured *in vivo* by means of intracellular recordings in the rat brain, synaptic responses are almost undetectable in layers II&III (Stern et al, 2001). At P14, evoked sensory synaptic potentials and action potentials (APs) have appeared in layers II&III neurons and layers II&III receptive fields have a mature organization. Thus, the development of layers II&III receptive fields is coincident with the period at which those neurons begin to respond to presynaptic activity and at which rats begin to use their whiskers for exploratory behaviour (Welker, 1964), which suggests that the fine wiring of intracortical circuitry might be experience-dependent. Indeed, sensory experience suppression induced during the second postnatal week in rats by depriving all whiskers for 5 days disrupts normal receptive field maturation in layers II&III with a critical period around P14 (Lendvai et al., 2000; Stern et al., 2001). The second week of the postnatal development is also characterized by synaptogenesis and a high turnover and motility of spines, tiny protrusions from dendrites receiving synapses (Micheva and Beaulieu, 1996; Lendvai et al., 2000). Thus, the establishment of layers II&III receptive fields should involve structural synaptic plasticity.

Developmental plasticity at layer IV - II&III synapses. In the mature cortex, layer IV to layers II&III projections from spiny stellate and pyramidal neurons are mainly restricted to the home barrel column (Harris and Woolsey, 1983; Lubke et al., 2000; Petersen and Sakmann, 2001). At P8, axonal topography is less columnar in rats: layer IV neurons innervate substantially neighbouring columns (Bender et al., 2003). It is thought that the precision of cortical connections is improved by activity-dependent refinement of axons during postnatal development (Lowel and Singer, 1992; Katz and Shatz, 1996). Activity-dependent competition between layer IV inputs to layers II&III might drive the refinement of layer IV - II&III projections, shaping the precise functional topography of the cortical column in which almost all neurons respond preferentially to the corresponding whisker.

The potentiation of responses to the spared whisker in layers II&III of the deprived barrel columns following univibrissa rearing from P6 could originate from structural plasticity at layer IV - II&III projections (Fox, 1992). It is possible that based on a normal pattern of layer IV receptive fields, the disequilibrium between activity in the spared and deprived pathways alters the columnar segregation of layer IV - II&III projections via abnormal growth and/or retraction of axons, leaving aberrant axonal branches from the spared barrel neurons in neighbouring deprived barrel columns. The increased cortical representation of the spared whisker after univibrissa rearing could then be due to impaired columnar segregation of layer IV - II&III projections. Deprivation of all whiskers during the second postnatal week does not prevents the establishment of a columnar topography of those projections (Bender et al., 2003). However, reconstructions of layer IV axonal projections following univibrissa rearing, a procedure that should drive competition for cortical space, rather than after all whisker deprivation could lead to different results.

Alternatively, using laser scanning photostimulation, Shepherd and colleagues have demonstrated that the broadening of neuronal receptive fields following all whisker deprivation between P9-P14 is concomitant with a decrease functional connectivity of layers II&III with barrel hollow region and an increase connectivity of layers II&III with barrel septae (Shepherd et al., 2003). They propose that lemniscal and non-lemniscal projections from barrel and septal neurons compete to drive layers II&III neurons and that experience favors lemniscal projections with their precise and synchronized inputs.

In addition to rearrangements of axonal arbors, plasticity could involve modulation of existing synapses. Increasing number of evidences indicate that LTP and LTD-like mechanisms could be engaged in experience-dependent plasticity at excitatory layer IV - II&III synapses in the barrel cortex as well as in the visual cortex *in vivo* (Finnerty et al., 1999; Shepherd et al., 2003; Allen et al., 2003; Takahashi et al., 2003; Heynen et al., 2003; Celikel et al., 2004). LTP and LTD of layer IV to layers II&III pathways in the barrel cortex of neonatal animals are induced following timing-based induction protocol *in vitro* (Feldman, 2000) and using theta-burst stimulation of layer IV *in vivo*

(Glazewski et al., 1998b). For instance, LTD-like mechanisms could explain the depression of PW responses in layer II&III of deprived barrel column adjacent to spared barrel columns (Fox, 1992; Rema et al., 2003). Several *in vitro* cortical slice experiments have suggested that precise temporal associations between EPSPs and postsynaptic action potentials induce modulations of synaptic strength (Markram et al., 1997; Feldman, 2000; Allen et al., 2003). LTP is induced at layer IV to layers II&III synapses when EPSPs lead APs in the postsynaptic neurons within a narrow window while LTD occurs when APs lead EPSPs within a broader window (Feldman, 2000). Given that the temporal window is longer for inducing depression than for inducing potentiation, spontaneous activity in the deprived pathway might drive depression if it is poorly correlated with postsynaptic activity. As a neuron in a deprived barrel column will tend to be driven by the spared pathway, this model predicts the depression of the deprived input (Celikel et al., 2004). In agreement with that, Allen and colleagues have shown that whisker deprivation for 9-21 days beginning at P12 causes measurable reduction in the amplitude of the EPSPs evoked in layers II&III by stimulation of layer IV in deprived columns as compared to non-deprived columns of rats (Allen et al., 2003). Interestingly, they furthermore have demonstrated that whisker deprivation occludes subsequent *in vitro* induction of LTD while enhancing LTP in deprived columns, as it would be expected if LTD had induced the synaptic transmission weakening observed *in vivo*.

Developmental plasticity at transcolumnar connections in layers II&III. Receptive fields in layers II&III crucially depend on intracortical connections between barrel columns (Armstrong-James et al., 1992). The second week of the cortical development is characterized by a growth of axonal projections across barrel columns in layers II&III (Mccasland et al., 1992). In contrast with TC-projections, intracortical projections as examined by horse-radish peroxydase (HRP) or cholera toxin B subunit (CTB) injections into supragranular layers are drastically diminished in barrel cortices when the infraorbital nerve is sectioned on P7 or following neonatal clipping from P0 (Mccasland et al., 1992; Keller and Carlson, 1999). This observation indicates that the development of intracortical circuits is dependent on sensory experience and is susceptible of large scale activity-dependent rearrangements.

Functional reorganization of horizontal pathways within layers II&III has been observed following deprivation of selected rows of whisker while sparing others beginning from P10 (Finnerty et al., 1999), suggesting that the potentiation of responses to the spared whiskers in deprived barrel columns may also rely on an increase of transcolumnar transmission. Functional and anatomical experience-dependent reorganizations of horizontal cortical networks were demonstrated in the visual cortex of neonates and adults cats (Callaway and Katz, 1991; Darian-Smith and Gilbert, 1994) and the somatosensory cortex of adult rats and monkeys (Florence et al., 1998; Hickmott and Merzenich, 2002).

III.2. Cortical plasticity induced by the specific deprivation of a set of whiskers in adults

Cortical plasticity in adults was originally demonstrated following injury to a peripheral nerve: the somatosensory cortical region that loses its sensory input from peripheral receptors begins to respond to neighbouring intact receptors (Merzenich et al., 1983; Merzenich et al., 1984). This "invasion" of a deprived cortical territory by spared inputs in adults was then also shown in the auditory cortex following partial cochlear lesion (Robertson and Irvine, 1989) and in the visual cortex following restricted retinal lesions (Kaas et al., 1990; Chino et al., 1992). Intracortical mechanisms are thought to be responsible for the topographical reorganization of visual cortex following retinal damage: receptive fields of neurons near the border of the deprived area immediately expand seemingly through the potentiation of existing subthreshold inputs (Gilbert and Wiesel, 1992), while intracortical axonal sprouting mediates long-term reorganization of functional topography by months after the lesion (Darian-Smith and Gilbert, 1994). Similarly in the adult barrel cortex of rodents, the destruction of a set of whisker follicles results in the expansion of spared whisker cortical representations in adjacent cortical regions that had lost their sensory inputs, i.e. deprived cortical regions, as assessed using 2-deoxyglucose (2-DG) imaging of neuronal activity (Kossut et al., 1988). Although studies from the visual cortex strongly support the view that horizontal connections mediate functional and anatomical cortical reorganization, several other mechanisms were implicated including activity-dependent changes in the balance between excitation and inhibition or plasticity at TC-afferents and feedback cortico-cortical connections (Welker et al., 1989b; Jones, 1993; Florence et al., 1998; Krupa et al., 1999).

Potentiation of spared whisker responses

The barrel cortex has also been shown to exhibit robust receptive field plasticity in response to transient innocuous alterations in the pattern of whisker experience in adults animals (about two month-old in mice and six month-old in rats) by selective whisker deprivation through either clipping or trimming (Delacour et al., 1987; Diamond et al., 1993; Armstrong-James et al., 1994; Glazewski et al., 1996; Barth et al., 2000; Trachtenberg et al., 2002). The effects of whisker deprivation show some commonalties with those induced by lesions to peripheral receptors or sensory pathways: neurons in barrel columns corresponding to deprived whiskers acquire enhanced responses to the spared SWs in both adult mice and rats (Glazewski et al., 1996; Glazewski et al., 1999; Lebedev et al., 2000; Trachtenberg et al., 2002). In accordance to that, it has been shown in adult rodents using 2-DG, optical imaging techniques and single unit recordings with microelectrode array, that the functional representations of the spared whiskers in the cortex could expand in a use-dependent manner in deprived adjacent cortical regions (Kossut, 1998; Polley et al., 1999; Lebedev et al., 2000). In addition to changes in the deprived cortex, neurons within the adjacent non-deprived cortex also exhibit experience-dependent plasticity. Following

whisker pairing in rats, neurons in a barrel column corresponding to a spared whisker exhibit a potentiated responsiveness to their PW and to the other spared whisker (Diamond et al., 1993; Armstrong-James et al., 1994; Diamond et al., 1994; Lebedev et al., 2000). On the contrary, responses to deprived whisker in spared barrel column decrease, this depression being accentuated with the duration of the whisker pairing period (Armstrong-James et al., 1994). Accordingly, as assessed after the trimmed whisker has regrown, neurons show a bias in their responses to the other spared whisker in comparison to responses towards an adjacent deprived whisker.

Cortico-cortical versus thalamocortical synaptic plasticity

Plasticity at intracortical transcolumnar connections. Following 18 days of single whisker sparing in adult mice, the potentiation of responses towards the spared whisker in the deprived barrel columns occurs in layers II&III but not in layer IV (Glazewski et al., 1996; Glazewski et al., 1999), suggesting that the main locus for experience-dependent plasticity in the adults may reside at cortico-cortical synapses. Similar observations have been made in rats in which receptive fields of layers II&III neurons exhibit plasticity upon a period of single whisker sparing beyond P28 whereas layer IV neurons not (Glazewski and Fox, 1996). Potentiation of responses to the spared whisker in barrel columns corresponding to deprived whisker therefore results presumably from intracortical plasticity in adult animals, i.e. via the potentiation of transcolumnar synaptic connections from the spared to the deprived columns in layers II&III, rather than from subcortical or thalamocortical plasticity [1].

In adult rats, plasticity can be induced in layer IV using the whisker pairing paradigm in which all but 2 adjacent whiskers are removed. Following 3-10 days of whisker pairing, neuronal responses in layer IV of a barrel column corresponding to a spared whisker towards the other spared whisker potentiate (Diamond et al., 1993; Armstrong-James et al., 1994). This modification only affects the late component of evoked firing (10-100 ms poststimulus) and is therefore also likely due to a potentiation of transcolumnar synaptic transmission between barrel columns rather than to modifications at subcortical or thalamocortical levels (Armstrong-James et al., 1994).

Intracolumnar plasticity. In adult mice, it has been shown that responses to the spared whisker also potentiate in layers II&III but not in layer IV of the corresponding barrel column after 16 hours of single sparing, suggesting a potentiation of layer IV – II&III synapses (Barth et al., 2000). In adult rats, potentiation of spared whisker responses in the corresponding layer IV barrels, i.e. PW potentiation, occurs following 3 days of whisker pairing (Diamond et al., 1993; Armstrong-James et al., 1994), however in layers II&III, similar modifications occur within only 24h hours (Diamond et al.,

[1] In the adolescent rats (~P45), it should be noted that using a chessboard deprivation pattern, potentiation of spared whisker responses in deprived barrel column is also substantial in layer IV (Wallace and Fox, 1999). The authors argue that layer IV plasticity is similarly consistent with a model whereby inter-columnar connections potentiate.

1994). After 3-10 days of modified sensory experience, the PW potentiation in layer IV of the spared barrels does not affect the first 10 ms of the post-stimulus time epoch (Armstrong-James et al., 1994) suggesting modulations of intracortical synaptic connections within a barrel.

Thalamocortical plasticity. When the duration of modified experience is prolonged up to 30 days in the adult rat, receptive field alterations in spared barrels also concern the first milliseconds of the PW-evoked responses and could even involve the creation of new early evoked discharges to a spared SW (Armstrong-James et al., 1994; Wallace et al., 2001). The potentiation of the early discharges evoked by the PW suggests a strengthening of TC-synaptic inputs from the corresponding barreloid. The novel, short latency responses to a spared SW could be due to the strengthening of the small proportion of TC-afferents that diverge to terminate in the barrels surrounding the main target of this whisker (Jensen and Killackey, 1987; Arnold et al., 2001; Hage, 2003).

Mechanisms of plasticity in adults

Hebbian-like modulations of synapses. Since they lost their main sensory input, deprived barrel columns will be less activated during the period of selective whisker removal. At the same time, because a subset of whiskers is absent, the remaining whiskers will be more powerfully engaged for environment exploration, and neurons in barrel columns corresponding to spared whiskers will be activated stronger than in control conditions. Moreover, surround inhibition from adjacent whiskers will be attenuated in non-deprived barrel columns close to deprived ones (Kelly et al, 1999). Therefore the selective removal of a subset of whiskers creates a combination of deprivation and stimulation in the barrel cortex and induces competition associative interaction between sensory inputs. The potentiation of spared inputs in deprived barrel columns (Glazewski et al., 1996; Glazewski et al., 1999) could be interpreted as an Hebbian-like strengthening of synaptic inputs from sensory driven barrel columns to barrel columns that had lost their main sensory input (Hebb, 1949). At the same time, the Hebbian models (Stent, 1973; Bienenstock et al., 1982; Cruikshank and Weinberger, 1996) predict that deprived inputs on neurons in barrel columns corresponding to spared inputs will be functionally weakened. In agreement with that, deprived whiskers were shown to evoke reduced responses into barrel columns corresponding to spared whisker (Armstrong-James et al., 1994). It has been proposed that following Hebbian models, the preferred use of a pair of whisker potentiates reciprocally the spared inputs between the corresponding barrel columns (Armstrong-James et al., 1994). Whisker pairings should result in an enhanced overlap of adjacent spared whisker representations, as for syndactyly monkeys (Clark et al., 1988), and an enhanced correlations of neuronal firing between spared barrel columns (Lebedev et al., 2000). It has been also suggested that PW-potentiation following whisker pairing may results from a co-operative effect of the spared inputs that potentiate together in a Hebbian manner, leading to strengthening of transmission within neurons of

corresponding barrels and between layer IV neurons and their layers II&III counterparts (Armstrong-James et al., 1994; Diamond et al., 1994). In addition, neurons in spared barrel columns presumably lose surround inhibition from deprived inputs, which could explain why PW responses are strengthened even if a single whisker is spared (Barth et al., 2000).

In the adult barrel cortex, the experience-dependent potentiation of spared inputs in adjacent barrel columns that had lost their main sensory inputs presumably involves plastic changes in the properties of inter-columnar connections (Diamond et al., 1993; Armstrong-James et al., 1994; Glazewski et al., 1996). In agreement with that, Lebedev et al (2000) have shown using cross correlation analysis of neuronal spiking that after 3 days of whisker pairing, the effective connectivity is higher between neurons of paired barrel columns versus neurons of unpaired ones. As mentioned above, functional reorganization of horizontal pathways has also been observed indirectly in the barrel cortex of young rats following 10 days of selective whisker deprivation beginning at P11-15 (Finnerty et al., 1999). In adult mice, activity-dependent plasticity of field potentials has been noticed at horizontal pathways between barrel columns *in vitro* (Urban et al., 2002) and between the inner border of a barrel and layers II&III of neighbouring barrel columns *in vivo* (Glazewski et al., 1998a). The induction of LTP of synaptic potentials in the barrel cortex is usually restricted to neonatal animals (Feldman et al., 1999; Feldman, 2000). To our knowledge, direct evidences of LTP at intracortical synapses are sparse in the adult sensory cortex (Aroniadou-Anderjaska and Keller, 1995). However similar mechanisms might play a role in adults as suggested by several lines of evidences. Potentiation of responses following whisker pairing are dependent on the activation of NMDA-Rs within the cortex, as infusing D-APV during the period of pairing completely prevents the bias in SRF to occur (Rema et al., 1998). NMDA-Rs are likely key actors in the induction of activity-dependent modifications because of the slow kinetics of the NMDA-R mediated postsynaptic potential that confer them the capacity to act as coincidence detectors, and because they mediate postsynaptic Ca^{++} influx which is necessary for changes in synaptic strength (Cull-Candy and Leszkiewicz, 2004 for a review). It has been shown that the induction of LTP *in vitro* depends on NMDA-R currents (Harris et al., 1984; Artola and Singer, 1987; Lee et al., 1991). Potentiation of the spared whisker is prevented in adult homozygous mutant mice bearing point mutation into the auto-phosphorylation site of the CaMKII gene which is known to be necessary for the induction of LTP *in vitro*, presumably through the phosphorylation of AMPA-Rs (Glazewski et al., 1996; Glazewski et al., 2000). Moreover, the level of potentiation is decreased in knockout mice for the cyclic adenosine monophosphate responsive promotor element (CRE) binding protein (CREB); a molecule regulating the transcription of effector's genes that could be implicated in plastic changes (Glazewski et al., 1999). Sparing a single whisker induces an increase in CRE-mediated gene expression in the corresponding barrel column (Barth et al., 2000). CREB has been shown to be necessary for LTP

maintenance and memory (Fox, 2002 for a review). Altogether, altered sensory experience in adult mice induces plasticity at intracortical synaptic connections following mechanisms that might share some commonalties with LTP synaptic plasticity *in vitro* since the induction of both phenomenon are dependent on the same molecules.

Glutamic acid decarboxylase (GAD) expression, reflecting the synthesis of the inhibitory neurotransmitter GABA, is regulated in an activity-dependent manner in the cortex (Hendry and Jones, 1986; Welker et al., 1989b; Welker et al., 1990; Akhtar and Land, 1991). GAD diminishes in deprived barrel within 3 days following selective ablation of whisker follicles in mice (Welker et al., 1989b). Trimming whiskers of adult rats leads to a reversible decrease of GAD immunoreactivity in deprived barrels (Akhtar and Land, 1991). The reduced GABA levels in deprived cortex may facilitate the potentiation of synaptic efficiency at horizontal connections from the spared cortex by enabling spared SW-evoked activity to generate high level of postsynaptic activity (Welker et al., 1990). Interestingly, Lebedev et al (2000) observed that after 3 days of whisker pairing, neurons in deprived barrel columns not only exhibit enhanced responses to the spared whiskers but also to some clipped whiskers within their SRF. Since these measurements were not made after whisker regrowth but using the short stub of the clipped whisker, the enhanced responses to deprived whiskers strongly indicate a disinhibition presumably due to a persistent low level of GABA in deprived barrels. The influence of disinhibition onto the induction of activity-dependent plasticity is further supported by the observation that the induction of LTP in slices preparations from the barrel cortex necessitates the blockade of GABA receptors (Feldman, 2000).

Morphological changes in intracortical connectivity. Using two-photon laser-scanning microscopy for imaging dendritic spines of layer V pyramidal neurons in layer I for several weeks, it has been shown that chessboard deprivation induces an increase in the turnover of spines (Trachtenberg et al., 2002). Electron microscopy of imaged spines reveals that sprouting and retraction of spines are associated with synapses formation and elimination. Therefore, morphological changes at the synaptic level may underlie the modifications in physiological response properties observed following modified sensory experience by the remodelling of neuronal circuits. Such remodelling could potentiate horizontal transmission via the regulation of the density of synapses made by already present inter-columnar axonal branches. At a larger-scale, structural rearrangements of horizontal connections through axonal sprouting have been documented in visual and somatosensory cortex of adult mammals following deafferentation (Darian-Smith and Gilbert, 1994; Florence et al., 1998). A study from our laboratory recently demonstrated the growth of new intracortical projections in the adult barrel cortex of mice following deafferentation from VB inputs: 5 weeks following a VB lesion, a deafferented barrel column receive more cortico-cortical projections including in layer IV, especially for long distance projections presumably originating from still afferented barrel columns (Croquelois et al., 2005). However it is

not known whether such large-scale anatomical changes could occur following an innocuous alteration in whisker sensory experience.

Depression of responses in adolescent rats

In adolescent rats (about 1-2 month-old), deprived cortical areas corresponding to chronically deprived whiskers acquire enhanced suprathreshold responses to neighbouring spared whiskers while responses to the deprived PW diminish (Glazewski et al., 1998b; Wallace and Fox, 1999). Such depression of PW responses in deprived barrel columns has not been observed in adult rodents. Some depression of cortical responses in deprived cortical area is already induced in the barrel cortex when all whiskers are removed (Glazewski et al., 1998b) indicating that depression in layers II&III could be homosynaptic. However, experience-dependent depression is greater when some active inputs remain and maximal in deprived cortical regions near a cortical area corresponding to an active input (Glazewski et al., 1998b; Wallace and Fox, 1999). In the same way that in the visual cortex where monocular deprivation causes far stronger depression of the closed eye responses than binocular deprivation (Wiesel and Hubel, 1965), activity in the spared whisker pathway appears to drive depression at the deprived input. In accord to that, chronic cortical application of muscimol prevents deprivation-induced depression in adolescent rats, indicating that depression of whisker responses depend on cortical activity and moreover does not simply result of a passive decay of synaptic strength following prolonged reduction of evoked activity (Wallace et al., 2001). Following single whisker sparing, depression of neuronal responses towards the PW in the deprived barrel columns occur in layers II&III but not in layer IV, suggesting that layer IV to layers II&III synapses could have been modulated by experience (Glazewski et al., 1998b).

III.3. Cortical plasticity induced by chronic whisker stimulation

Besides whisker deprivation, chronic whisker stimulation in normally behaving adult mice using the Lausanne whisker stimulator (Melzer et al, 1985) has also been used to evoke controlled changes in the sensory input to the barrel cortex. Chronic whisker stimulation differs from selective whisker removal paradigms, which evoke a combination of whisker deprivation and preferred usage of spared whiskers (see above). During chronic whisker stimulation, the corresponding barrel column receives a continuous increase in activity from its main sensory input. Accordingly, it has been shown that a 45 minutes period of continuous whisker stimulation yields an increase level of neuronal activity in the corresponding barrel column, as studied with deoxyglucose as a metabolic marker (Melzer et al, 1985). Although the chronic whisker stimulation could be regarded as far-removed from the normal mouse sensory experience, it is important to study how neuronal networks react to an increase in sensory stimulation if we have to understand experience-dependent neuronal plasticity. Chronic stimulation presents the advantage to preserve the functional integrity of all sensory inputs to the barrel cortex, which allows the animal to use them during the

stimulation protocol. Thus, the "normal" sensory information is not altered. Furthermore, experience-dependent changes in neuronal responses to sensory inputs can be tested immediately after the stimulation protocol.

Welker and colleagues (1992) have studied the pattern of deoxyglucose uptake in the barrel cortex of adult mice that were allowed to explore an enriched environment following a 24h period of chronic single whisker stimulation. They observed that the deoxyglucose uptake was lowered in the barrel columns corresponding to chronically stimulated whisker as compared to barrel columns in non-stimulated animals, indicating a decrease in neuronal activity in the stimulated barrel column. In another study, Welker and colleagues (1989b) have shown that 4 days of chronic whisker stimulation induces increase glutamic acid decarboxylase (GAD) immunoreactivity in the corresponding barrel columns, particularly prominent in layer IV. The enhanced expression of this biosynthetic enzyme of GABA suggests an increased release of this inhibitory neurotransmitter during the period of chronic stimulation. The increase in GAD immunoreactivity lasts for several days, diminishing gradually after the end of the stimulation. Although the increase in GAD immunoreactivity was observed after 4 days of peripheral stimulation, these results suggest that the decrease in neuronal activity demonstrated after 24h of stimulation is due to an increase in inhibition in layer IV, gating the peripheral sensory activity entering the cortical circuitry.

Using serial-section electron microscopy, another study from our laboratory has demonstrated that a 24h period of whisker stimulation induces morphological changes at the synaptic level: the density of spines is about 30 % higher in the stimulated barrel as compared with an adjacent, non-stimulated barrel, or with the similar barrel in a non-stimulated mouse (Knott et al, 2002). Spines are small dendritic protrusions of excitatory neurons receiving the vast majority of excitatory synapses (Harris, 1999). Accordingly, the total density of synapses increases, while the density of synapses with dendritic shafts remains unchanged. Using GABA immunohistochemistry to differentiate inhibitory of excitatory synapses, Knott and colleagues also revealed that both types of spineous synapses increase in density but that the proportional increase in inhibitory (4 fold) synapses is robustly higher. The ratio between excitatory and inhibitory synapses shifts from 4.59 in non-stimulated to 2.89 in stimulated animals. All spines that bear an inhibitory synapse always possess an excitatory synapse, forming so-called double-synapses spines (Micheva and Beaulieu, 1995; Knott et al., 2002). The insertion of new inhibitory synapses on spines, either pre-existing or newly formed, leads to a nearly 4 fold increase in the number of double-synapses spines. Strikingly, the total spine density and the density of excitatory synapses return to control levels 4 days after the chronic stimulation was halted but the density of inhibitory spineous synapses remains significantly higher. This study is the first demonstration of an increase in the density of cortical synapses following an innocuous and relatively short-lasting period of modification in sensory activity in the adult. Other evidences for use-dependent morphological plasticity at the level of the spineous synapse were previously reported following whisker deprivation from birth in

rats (Micheva and Beaulieu, 1995) and chessboard whisker deprivation for four days in the adult mouse (Trachtenberg et al, 2002). Therefore, spineous synapses represent a structural substrate of experience-dependent plasticity throughout life and may constitute the basis of the impressive memory-storage capacity of the cerebral cortex.

How does chronic stimulation lead to synaptogenesis? Regulation of the expression of genes and their products by synaptic events is one of the mechanism that could translate sensory experience into structural modifications. An attractive candidate is the brain derived neurotrophic factor (BDNF). The expression of BDNF is CRE-dependent (Shieh et al., 1998), and it has been shown that experience-dependent plasticity paradigms cause CRE-mediated gene expression (Barth, 1999). Welker and colleagues have observed an enhanced expression of BNDF mRNA (Rocamora et al., 1996) that peaks after 6 hours of chronic stimulation. Growth factors like BDNF are important for the development of inhibitory neurons (Marty et al., 1997; Kohara et al., 2003) and for activity-dependent synaptogenesis in organotypic cerebellar cultures (Seil and Drake-Baumann, 2000) and hippocampal slices (Marty et al., 2000). An increase in BDNF release could therefore promote and potentiate the role of GABAergic cells in cortical processing. Moreover, Genoud and colleagues have demonstrated in a heterozygous knock out BDNF +/- mouse strain that a reduced BDNF expression prevents the synaptic morphological changes induced by peripheral chronic stimulation in layer IV of the stimulated barrel, suggesting they are mediated by this neurotrophin (Genoud et al., 2004).

The increase in GAD immunoreactivity and in the density of spines with GABAergic synapses in layer IV and the decrease deoxyglucose uptake in the stimulated barrel strongly suggest that chronic stimulation activates a cortical mechanism resulting in a sensory habituation process by which the cortex regulates its level of excitation towards a behaviourally irrelevant stimulus. Are the drastic long-lasting changes at the synaptic level reflected in the response properties of barrel column neurons? In order to answer this question, the present study characterizes the physiological effects of prolonged sensory stimulation on the response properties of neurons in the barrel cortex. Part of this work has been published in Knott, et al (2002).

IV. Regulation of astrocytic transporters GLT-1 and GLAST

Thus, the chronic stimulation of a single whisker for relatively short periods of time (6-24h) induces robust experience-dependent changes in the cerebral cortex that can be observed at different level, from metabolism to regulation of gene expression and synaptic morphology, and constitute an unique tool to study cerebral plasticity mechanism in vivo. Astrocytes are known to maintain physiological concentrations of ions and neurotransmitters in extracellular space and to provide protection and energy to neuron. An increase number of studies tends to demonstrate that astrocytes are more than neuron passive partners and that they could play an important modulatory role in

neuronal transmission and plasticity (Araque et al., 2001; Bezzi and Volterra, 2001). Neurons and surrounding astrocytes should be considered as a functional interconnected entity, particularly at the level of the synaptic complex; therefore, it might be asked whether astrocytes undergo changes during synaptic plasticity events. Astrocytes can influence regulation of the glutamate in the extracellular space by spatially limiting the spread of glutamate and by capturing through glutamate transporters. Glutamatergic excitation through thalamocortical connections drives sensory cortex activation, constituting a central key factor in experience-dependent plasticity induction. Therefore, we decided to study whether the chronic stimulation alters the level of expression of astrocytic glutamate transporters, which would indicate that astrocytes are active partner in neuronal plasticity in the adult cerebral cortex.

Glutamate transporter function. Glutamate is not metabolized in the extracellular environment. The regulation of the level of glutamate outside cells depends only on cellular uptake by active transporter proteins in the plasma membrane of glia and neurons (Danbolt, 2001). Since synaptic clefts are in continuity with the extracellular space, the glutamate release at synapses may influence glutamate receptors outside synapses and at neighbouring synapses. Therefore, a strict regulation of extracellular concentration is required for a high signal-to-noise ratio in synaptic and extrasynaptic transmission. Furthermore, The relation between glutamate receptor activation and desensitization depends on the time course and amplitude of extracellular glutamate concentration (Barbour et al., 1994). Active translocation through transporters affect glutamate concentration in the synaptic cleft within a milliseconds timescale (Diamond et al, 1997, Grewer et al, 2000) and influences time-course of synaptic transmission as it was shown by prolonged excitatory postsynaptic current (EPSC) at parallel and climbing fibre synapses in cerebellum slices (Takahashi and Attwell, 1995; Overstreet et al., 1999). Besides this physiological, synaptic function, extracellular concentrations of glutamate in the brain must be strictly maintained at non toxic levels. Epileptic seizures and excitotoxicity are enhanced in knockout mice lacking the glutamate transporter GLT-1 (Tanaka et al., 1997).

Astrocytic glutamate transporters. So far, five different isoforms of plasma membrane glutamate transporters have been identified. They were named EAAT1 to EAAT5 for "Excitatory Amino Acid Transporters". They are differentially expressed in function of brain regions and cellular location. (Lehre et al., 1995; Lehre and Danbolt, 1998a). At least in the cortex, EAAT1 and EAAT2, usually termed GLAST and GLT-1, are the glutamate transporters mainly involved in the maintenance of physiological level of extracellular glutamate (Rothstein et al., 1996; Tanaka, K. et al, 1997).

GLT-1 is the major glutamate transporter, in terms of densities and glutamate uptake activity in several region of the brain, including thalamus, hippocampus, striatum and cerebral cortex (Tanaka et al., 1997). In the other regions of the brain, as the cerebellum, GLAST is the major transporter. Immunolocalisation studies revealed that GLT-1 and GLAST are expressed in astrocytes where they are localized at the plasma

membrane (Danbolt et al., 1992; Levy et al., 1993a; Torp et al., 1994; Rothstein et al., 1994; Lehre et al., 1995; Chaudhry et al., 1995). The two transporters are co-expressed in the same astrocyte. No studies have shown so far any astrocyte that was deficient in one of these transporters (Lehre et al., 1995; Tanaka et al., 1997). Their presence is undetectable in neurons of the mature brain. Density of transporters is higher in astrocytic membranes facing synaptic regions as opposed to regions of presumably lower glutamate release such as dendritic stems, pial surface, apposed astrocytic membranes and vascular endothelium (Chaudhry et al., 1995). These findings suggest that the localization of these transporters is carefully regulated and targeted to regions of high glutamate activity.

These transporters are able to catalyse the influx of one glutamate together with one proton and three Na+ in the exchange of one K+. They probably form homo-oligomers of two or three non-covalently connected subunits, each one formed of 8 transmembrane domains with intracellular C- and N-termini. Transporters are probably anchored in the membrane by proteins that could regulate their functions (for a review: Danbolt, 2001).

Regulation of glutamate transporters. The presence of neurons directly influences expression of GLAST and GLT-1 in astrocytic cultures (Perego et al., 2000). Decreasing neuronal activity by treating primary hippocampal cultures with the noncompetitive NMDA-R antagonist MK-801 or with Tetrodotoxin (TTX) reduce neuronal excitability and decrease the expression of both transporters (Perego et al., 2000). Both transporters are regulated during development and by several growth factors, pituitary adenylate cyclase-activating peptide (PACAP) and glutamate itself (Gegelashvili et al., 1997; Gegelashvili et al., 2000). During development, up-regulation of these transporters follows maturation of the brain: their levels dramatically increase during the most active period of synaptogenesis (Furuta et al., 1997; Ullensvang et al., 1997). Glutamate uptake activity in synaptosomal homogenate from brain tissue was shown to be upregulated by prior electrical stimulation of the brain (Nieoullon et al, 1983). Increase in glutamate uptake activity could be the result of postranslational regulation of the transporters, including translocation of transporters between intracellular storage compartment and plasma membranes; however it was observed that expression of GLAST and GLT-1 is highly regulated and so far no significant intracellular concentrations were detectable. (Casado et al., 1993; Levy et al., 1995; Poitry-Yamate et al., 2002; Levenson et al., 2002). A down-regulation of the expression of these transporters was observed in the striatum following deafferention (Levy et al., 1995; Levy et al., 1993b). However at present, little is known about the relationship between neuronal activity and glutamate transporter expression during normal brain function.

AIM OF THIS WORK

The present work investigates experience-dependent plasticity induced in the somatosensory cortex of adult mice by the chronic stimulation of a whisker during a 24h period in a freely behaving mouse. The main goal of this investigation was to analyse whether, and how, chronic whisker stimulation modifies the functional response properties of barrel cortex neurons, which would indicate experience-dependent adaptation in the way cortical networks process sensory information. Therefore, we assessed neuronal response properties at the end of the chronic whisker stimulation period in layer IV and layers II&III of the corresponding barrel column and of the neighbouring, non-stimulated, barrel columns. In all barrel columns, we quantitatively tested neuronal responses towards deflections of the PW as well as towards all directly adjacent SWs using extracellular recordings of local field potentials and single units AP firing under urethane anaesthesia. Neuronal responses in stimulated animals were compared with responses in non-stimulated animals. Long-term effects of chronic whisker stimulation were analysed in a group of mice recorded 4 days after the cessation of the stimulation. In order to study whether cortical neurons were altered in their sensitivity for the deflection of the chronically stimulated whisker or in their short-term dynamics, response properties of neurons towards PW-deflections of varying amplitude and frequencies were also compared between stimulated and non-stimulated mice.

Moreover, we addressed the effects of chronic whisker stimulation on the expression of astrocytic glutamate transporters GLT-1 and GLAST and of glutamine synthetase (GS), the main glutamate metabolising enzyme, in the stimulated barrel column using western blot analyses.

MATERIAL AND METHODS

I. Experience-dependent plasticity in the barrel cortex investigated with electrophysiological recordings

Recordings were made from a total of 160 adult female mice of the NOR strain derived from ICR stock (weight 25-35g, between postnatal days 60 and 90) (Van Der Loos et al., 1986). Sixty-seven mice were submitted to a 24h period of chronic stimulation of their left C2-whisker in the Lausanne whisker stimulator (see below) immediately before electrophysiological experiments (24h STIM). Eighteen mice received the same sensory treatment but were left to recover 4 days in their home cage before the recording sessions (4d-after-STIM). Seventy-five non-stimulated mice were used as control (NO STIM).

Chronic whisker stimulation

Prior to the chronic stimulation procedure, mice were anesthetized with Nembutal (sodium pentobarbital, 60 mg/kg, i.p.); a small ferrous metal rod (ion/nickel, 1.5 mm long, 0.2 mm in diameter, 0.6 mg) was then glued on the C2-whisker of their left whiskerpad, at aproximatively 3 mm of the skin surface. After recovery of the anesthesia, animals were placed for a period of 24h in the Lausanne whisker stimulator. This apparatus was previously described in details (Melzer et al., 1985); it consists of a cylindrical cage of 12.6 cm in diameter, surrounded by an electromagnetic coil. The coil delivers magnetic field bursts at 9 Hz, driving the movement of the ensemble whisker-metal (Burst duration: 40ms; intensity: $7X10^3$ A/m, rms value; frequency during burst: 50 Hz, from the mains). The maximal amplitude of the movement is fixed by the intensity of the magnetic field change and was chosen such that the stimulated whisker did not touch surrounding ones along its passive course. Mice had access to food and water and behaved freely. At the end of the stimulation period, the animal was removed from the stimulator and the metal rod was detached from the whisker with acetone under visual control by means of a dissection microscope.

Anesthesia and surgery

Electrophysiological recordings were carried out under urethane anesthesia (10% solution in distilled water; 2 mg/g body weight, i.p.; Sigma, CH). After the urethane injection, the animal was left for 30 minutes in a standard cage. It was subsequently

mounted in a stereotaxic frame equipped with a home-made headholder (Kucera, 1970) providing a continuous flow of oxygen in front of their nose. The mouse then received a subcutaneous dose of lidocain (0.1 ml; 1% w/v; Xylocain, ASTRA, CH) above the parietal bone. Body temperature was maintained at 37°C by a rectal thermistor-controlled heating pad (Homeothermic Blanket System Harvard 50-7061, England). Under optical magnification by means of a dissection microscope (Zeiss S21, DE), the scalp was incised and a craniotomy (~0.3X0.3 cm) of the right parietal bone was realized with a small electric drill to expose a large part of the somatosensory cortex including the posteromedial barrel subfield region (PMBSF). The exposed region was covered with 1% agar dissolved in 0.9% saline. The depth of the anesthesia was continuously controlled using several criteria: absence of whisking or eye blink reflex and burst rate of layer V cortical neurons between 2 and 4 Hz (Armstrong-James et al, 1985; Fox and Armstrong-James, 1986). Supplementary urethane doses (10% of the original dose) were given when necessary, based on these criteria.

Electrophysiological recordings

Extracellular single units spike discharges and local field potentials (LFPs) recordings.
The extracellular cerebral signal was recorded in cortical barrel columns using carbon fiber microelectrodes of low impedance (1-5 MΩ) that were fabricated as follows: a carbon fiber was insulated in a borosilicate glass pipette such that a 7 µm length tip comes out the pipette at its extremity (Armstrong-James and Millar, 1979), the tip was then sharpened by spark-etching (Armstrong-James et al., 1980). The microelectrode was fixed to a micromanipulator and lowered in the cortex normal to the pial surface. A reference electrode was placed at the level of the scalp. The differential voltage signal was recorded through a Neurolog headstage (NL100 AK, Digitimer Ltd, UK). Using a Neurolog modules arrangement (Neurolog system, Digitimer Ltd, UK), this signal was first amplified (2K) then filtered for extracellular spikes activity and local field potentials (LFPs) through parallel band-pass filters of 0.8-5 kHz and 0-45 Hz respectively. Single units spikes profiles were isolated based on their extracellular amplitudes and waveforms by means of a waveform time window discriminator that provided digital output of the discriminated spike (DIS-1, BAK electronic INC., USA). Amplified signal and time window were displayed on a digital oscilloscope (HM 408, Hamey GmbH, DE). An audiomonitor (AM8, GRASS, USA) was coupled to the recording amplification circuit. The audio reproduction of the electrophysiological signals allows to “listen” for patterns of voltage changes, which facilitate the mapping of cortical receptive fields and the monitoring of anesthesia depth. Digitized spike times and analog LFP waveforms were processed using the CED-Power 1401 interface (Cambridge Electronic Design, UK). Digitized spike times were recorded with a time resolution of 10 µs. LFP analog signal was sampled at 1 kHz.

Localization of recording sites. Data were recorded from barrel columns C2, C1 and D2 (see figure 1). Sample areas were identified using stereotaxic coordinates and cortical responses in layer IV to manually deflected contralateral whiskers. Indeed, the principal whisker (PW), corresponding to a specific cortical column, elicits the strongest response at the shortest latency in layer IV (Welker et al., 1993). The electrode was advanced using a three-dimensional micro drive (10 µm accuracy in each direction; Narashiga, Japan). Subpial depth of each recording site was precisely recorded. The initial position of the electrode in the vertical axe was determined both by visualization of the black tip of the electrode through a dissection microscope and by the typical decrease in electrical noise when contact was achieved with the pia matter. For the single unit analysis, recording sites were targeted to supragranular layers II&III and granular layer IV; the allocation to a cortical layer was based on vertical position measurements and previously determined cortical layer boundaries for NOR strain (Welker et al., 1993): layers II&III= 150-350 µm from pial surface, layer IV= 350-480 µm. Three to 8 neurons were recorded during a single microelectrode penetration and each recorded unit was separated from the other by a minimal distance of 50 µm along the depth. One to 3 penetrations in barrel column C2, C1 and/or D2 were achieved in each animal, separated tangentially by a minimal distance of 100 µm. For further confirmation of the identity of the recorded barrel column, each penetration was marked at the end of the recording session by a small electrolytic lesion made 400 µm from pial surface by passing a 1.6 µA negative current during 7 seconds through the microelectrode (see below).

Data analyses

Single units activity. Analyses were carried out using home made scripts written in Spike 2 software language (Cambridge Electronic Design, UK). Spike times were collected into 1 msec bins in two separate channels, retrieving either all spike times following each whisker deflection to build peristimulus time histograms (PSTHs) or only the time of the first spike after stimulus onset to build latency histograms (LHs). All whisker evoked responses of isolated units were recorded upon a minimum of 50 repetitions of the whisker deflection.

Quantification of the neuronal response was processed from PSTHs by the computation of the number of spikes count during specific post-stimulus time intervals. The response magnitude (RM) of a cell upon deflection of a whisker was defined as the mean number of spikes per stimulus evoked between 3 and 100 ms after the stimulus onset and corrected for spontaneous activity that was determined from the pre-stimulus 100 ms spike count. Time epoch analyses of the neuronal response to whisker deflection were realized by dividing PSTHs in 5 consecutive post-stimulus time intervals. These time epochs were chosen based on the temporal profile of the PSTHs of layer IV neuronal response to PW-deflection (see results section for determination of these epochs). Based on the LHs, modal latencies (Armstrong-

Jamesand Fox, 1987) and median latencies and IQ range (Welker et al., 1993) were calculated with a precision of 1 ms.

Local field potential analyses. Despite variations between individual traces, we noticed that after 25 whisker deflections the average LFP is very stable. In layer IV, whisker deflections evoked a multiphasic LFP characterized by a reliable short latency negative going phase followed by a positive phase (see figure 2.A). The following temporal measurements were based on the LFP waveform recorded at the level of layer IV and averaged upon 50 whisker deflections: onset time point of the initial negative going phase, time-to-peaks of both the initial negative and first positive phase relative to the whisker deflection. To calculate the onset time point of the initial component of the LFP, the first point of the first 5 poststimulus consecutive negative going bins (0.1 ms width) was tracked. The peak amplitudes of both negative and positive phases were computed as the differences between the voltage values at the onset time point and at the respective peaks. However, the voltage amplitude of the LFP signal could vary widely from one recording session to another; therefore, amplitude values from LFP waveforms were only used as relative values and were expressed in percent of the maximal voltage amplitude recorded within individual session.

Current source-density analyses. To calculate current source-densities (CSD), PW-evoked LFP waveforms were recorded in the corresponding barrel column with one microelectrode successively placed at regular intervals (Δz= 50 µm) from the pial surface to 1000 µm beneath, encompassing the entire thickness of the cortex. At each depth, the LFP waveform was averaged upon a minimum of 50 whisker deflections processed with the standard protocol (1.43° upward, 0.5 Hz; see whisker deflection procedure). These laminar LFP waveforms were used to calculate the second spatial derivative of potentials along the depth, which is referred as the one-dimensional CSD and yields spatiotemporal profiles of extracellular current sinks and sources. Those CSD traces were calculated in one dimension (z), with the assumption that currents in the x and y directions were negligible and that intracortical conductivity was constant, using the following formula: $CSD = -[V(z + \Delta z, t) - 2V(z, t) + V(z - \Delta z, t)]/\Delta z^2$; where $V(z, t)$ is the measured voltage at a subpial depth z (t= time; Δz= 50 µm) (Nicholson and Freeman, 1975; Mitzdorf, 1985). Finally, colour-coded contour plots which display current sources and sinks variations in function of the cortical depth and time were computed from linear interpolation between laminar CSD traces. CSD calculations were carried out using home-made scripts written in Matlab software (Mathworks, Inc., USA).

Whisker deflection procedure

To determine neuronal responses towards a sensory stimulus, individual whiskers were deflected one at a time, under urethane anesthesia. The whisker stimulus consisted of an upward deflection of 3 ms duration. The movement was applied through a piezoelectric stimulator designed according to Armstrong-Jamesand Fox (1987). To

this end, the whisker was first trimmed to ~15 mm length and inserted into a thin borosilicate glass tube attached to a piezoelectric bimorph slab. The tip of the glass probe was advanced at a distance of 10 mm of the mouse face. The upward movement of the probe was evoked by square wave voltage pulses delivered by a stimulator with high voltage output (Digitimer stimulator D59A; Digitimer Ltd, UK) gated by the CED-Power 1401 interface. Using the dissection microscope, we assured that the glass probe did not touched adjacent whiskers along its movement. Field potentials were carefully observed to make sure that deflections were applied only during appropriate stages of slow-wave sleep (Armstrong-James and Fox, 1988). Different deflection-amplitudes and frequencies were used for this study:

Standard protocol. In a first series of experiments, single unit responses evoked by the deflection of principal whisker and surround whiskers were quantified in the C2-barrel column and in the adjacent C1- and D2-barrel columns. We used a whisker deflection protocol that consisted of an upward deflection of 250 µm. This resulted in an angular displacement of the whisker of 1.43° from the horizontal plane (rise and fall times ~ 0.8 ms; duration: 3 ms). These deflection parameters correspond to those used in previous studies in our laboratory and elsewhere (Armstrong-James et al, 1987, 1988; Welker, 1993). This deflection-amplitude was sufficient to elicit maximal PW-evoked responses of layer IV neurons. Evoked responses of barrel column neurons were all recorded towards their PW and 6 to 8 immediate surround whiskers. Each individual whisker was deflected 50 times, at a frequency of 0.5 Hz to prevent steady state adaptation of neuronal responses. The same protocol was used during LFP recordings for the CSD analyses.

Varying amplitude protocol. In this second protocol, we recorded the responses of layer IV neurons in the C2-barrel upon a set of 7 different whisker deflections of 3 ms in duration varying in the amplitude of the movement: 0.06 °, 0.18°, 0.30°, 0.68°, 1.02, 1.43° and 2.1°, upward from the horizontal plane. Each of these different deflections was repeated 50 times at 0.5 Hz. The amplitude of the upward movement of the stimulation probe was dependent on the voltage amplitude of the square pulses applied to the piezoelectric stimulator. Before each recording session, the stability in the relation between voltage and upward amplitude was checked. The whisker was inserted in the glass probe so that it was immediately engaged by deflection onset. Moreover, the distance from the probe to the base of the whisker was carefully controlled in order to use the same angles of whisker movement between trials. Using our stimulator, both the amplitude and the velocity of the deflection were concomitantly decreased with decreasing voltage pulses as illustrated in Armstrong-Jamesand Fox (1987). Here we did not measure the actual variations of the velocity.

Varying frequency protocol. In the third deflection protocol, the PW was deflected at various frequencies (0.5, 1, 2, 4, 8 and 16 Hz) using the same angular displacement than with the standard protocol (1.43° upward). Blocs of ten deflections were used for each frequency and applied with a 2-seconds deflection-free interval between blocs.

Five runs of pseudo-randomly interleaved blocs were performed, resulting in a total of 50 deflections for each frequency.

Electrolytic lesions and histology

As mentioned above, microlesions (25 to 50 µm in diameter) were made in layer IV of all penetrations at the end of each recording session. Lesions were induced by passing a 1.6 µA negative current during 7 seconds through the microelectrode. Animals received an i.p injection of 0.1 ml sodium pentobarbital (5 mg) and were then transcardially perfused with 10% formalin in 0.9% NaCl. The brains were removed from the skull and post-fixed in the same fixative for 1 to 3 weeks. After 24h of cryoprotection in 30% sucrose (in phosphate buffer, 0.1M), the right hemispheres were sectioned tangentially (serial sections of 40 µm) to the pial surface overlying the barrel cortex on a cryotome. To achieve the orientation of the cutting plane, the hemispheres were separated, oriented using a guillotine (Rice and Anders, 1977) and mounted on a home-made plexiglas support. Sections were then stained for Nissl substance with cresyl-violet in order to visualize the arrangement of layer IV barrels. Reconstructions of the hemispheres allowed the histological identification of the recording sites. This identification was used to classify data for further analysis.

Statistics

Single unit responses to whisker deflection applied with the standard protocol. Single units data were classified by layers (layers II&III and layer IV), barrel columns (C2, C1 and D2) and experimental groups (NO STIM, 24h STIM, 4d-after-STIM). Within each class and for each whisker deflected, the distributions of the different response parameters (RM, modal and median latencies, time epochs activity) were tested for normality (Shapiro-Wilk test). Normality was rejected in half of the cases; therefore within groups comparisons were processed with parametric (one-way ANOVA) or non-parametric (Kruskall-Wallis) statistics. Two other aspects of the data were taken into account in order to build a robust statistical model for the comparisons between groups. First, for each response parameter, the values calculated towards the different individual whiskers had to be considered as dependent variables. Second, inter-animal variability had to be integrated. To allow appropriate statistical analyses, data were therefore normalized with a transform ranking method before entering a multivariate hierarchical model ("group animal (group)"). For each variable, the transformation explained variability of the samples at $p<0.01$ and the model was validated. For each parameter of the response, the hypothesis of no overall group effect on the cluster of dependant variables was tested with a MANOVA test criteria (Wilk's lambda), in each layer and barrel columns. Where the null hypothesis was rejected, multiple comparisons of each variable between groups could then be performed with minimized risks of false positive (Type II error) using Tukey's studentized range (HSD; honestly significant difference) tests, which took the inter-animal variability as a potential error

source. For comparisons of time epochs activity between groups, a SAS GLM procedure of repeated measures analysis of variance with contrast variables was used (SAS Institute Inc., USA). As a supplementary confirmation, each observed significant differences between groups was shown to be also significant at $p<0.01$ without the data transformation using one-way ANOVA or non-parametric Kruskall-Wallis test in function of the distribution of the variables.

Frequency and amplitude-dependent responses. For the series of recordings in which the PW was deflected at different amplitudes or frequencies, the relationships between deflection-amplitude or frequency and response parameters (RM, modal latency and time points of average LFP traces) were first tested with regression statistics and correlations t-tests. For the comparisons between groups, mean values of response parameters at each deflection-amplitude or frequency were compared using a multivariate analysis of variance (MANOVA) and Tukeys' tests. To test if the effect of varying the deflection onto neuronal responses was similar in 24h STIM and NO STIM groups of mice, a SAS GLM model of ANOVA with co-variable was built. This model allows the comparison of the effect of the deflection onto response parameters in the two groups (group*amplitude; group*frequency). The observed significant differences were verified with non-parametric statistics. All statistics were realized with SAS system for windows (SAS Institute Inc., USA).

II. Measurements of the effects of chronic whisker stimulation onto the expression of GLAST and GLT-1 protein in the barrel cortex

Levels of protein expression of the glutamate transporters GLAST and GLT-1 and of glutamine synthetase (GS) were analyzed in the barrel cortex of 62 adult female mice (weight 25-35g) from the ICR derived NOR strain (Van Der Loos et al., 1986) in relation to a period of increased sensory stimulation. A total of 40 mice were submitted to a 24h period of chronic single whisker stimulation. In 22 of these mice, a sample of the barrel column corresponding to the stimulated whisker was extracted immediately after the stimulation period (24h STIM group). The remaining 18 mice were placed back in their home cage for 4 days (4d-after-STIM group). Barrel column samples from 22 non-stimulated mice were used as control (NO STIM group).

Barrel column dissection for protein quantification

Anaesthesia and surgery. The dissections of barrel columns were made under Nembutal anaesthesia (100 mg/Kg body weight, i.p.). The craniotomy was conducted as explain above. The location and extent of the cortical representation of whisker C2 (C2-barrel column) was carefully mapped by recording multiunit activity towards manual deflection of the contralateral whiskers. Surface blood vessels were used as bench marks. A forceps-broke glass micropipette with an internal tip of 220-280 µm (corresponding to the diameter of the C2-barrel) was then placed normal to the pial surface above the mapped C2-barrel column, by the use of a calibrated three-dimensional microdrive (10 µm accuracy in each direction; Narashiga, Japan) and a dissection microscope. Dura matter was removed and penetration of the pipette was made perpendicular to the brain surface to a depth of 800-1000µm. Barrel column tissue was finally aspired through the pipette with a mini-pump. The prelevated tissue was washed in PBS 0.01M (pH 7.0) to remove excess of blood, transferred to buffer A (see below) and stored at -80°C. The whole process from the Nembutal injection to the storage of the barrel column sample in the refrigerator took 30-45 minutes.

Histology and verification of collected samples localization. Immediately after the barrel removal, the animal was transcardially perfused with 10% formalin in 0.9% NaCl. The brain was removed from the skull and postfixed in the same fixative for 1 to 3 weeks. After 24h of cryoprotection in 30% sucrose (in phosphate buffer, 0.1M), serial sections of 40 µm were cut on a cryotome tangentially to the pial surface overlying the barrel cortex, as described above. Sections were then Nissl-stained with cresyl-violet in order to confirm the localization of the dissected cortical sample and to estimate its radial and tangential extent. Figure 2 schematizes the barrel column dissection method. The right diagram shows an example of one mouse barrel cortex in which the C2-barrel column was aspired. This coronal view was based on the reconstruction of the serial tangential sections. Almost the entire barrel column C2 was

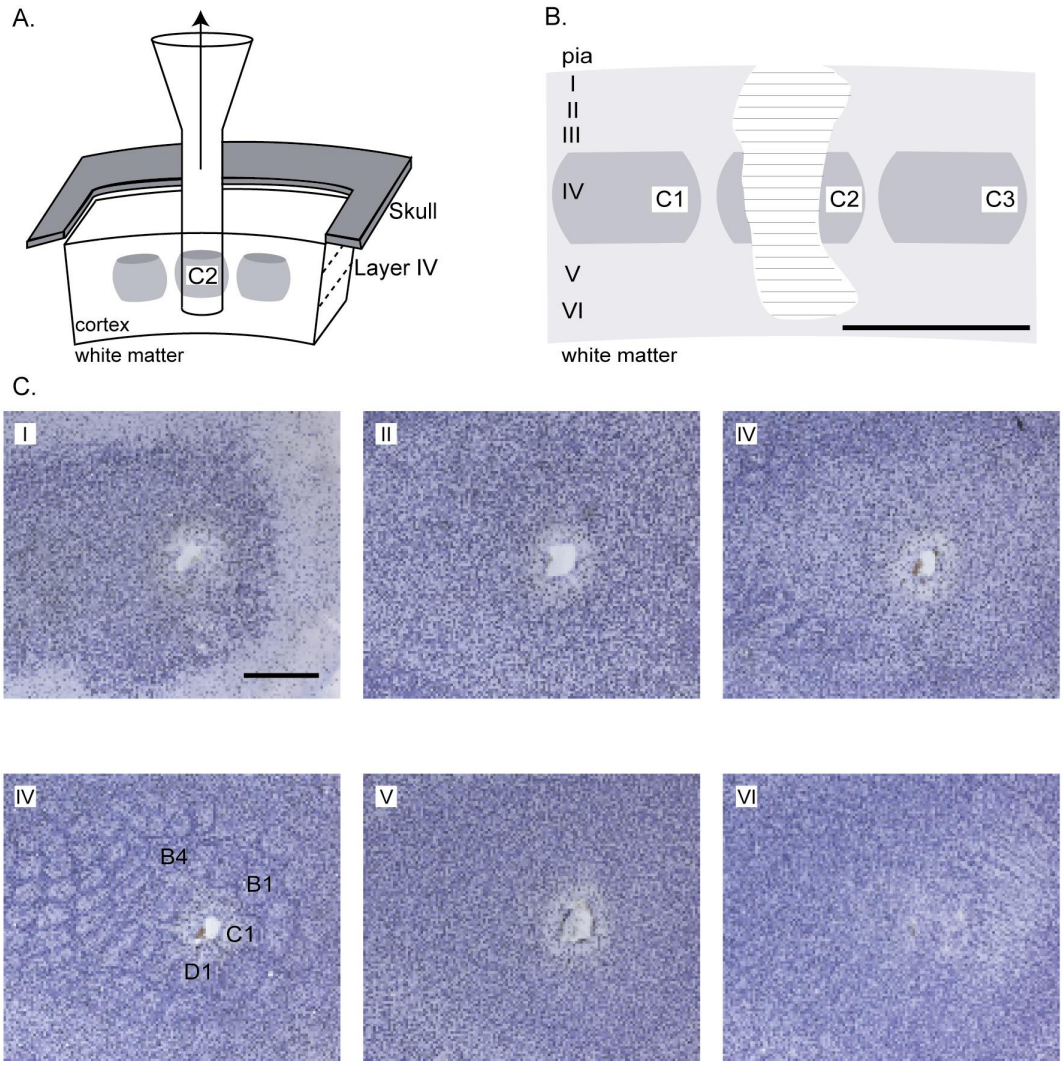


Figure 2. Barrel column dissection method.

(A) Under Nembutal anesthesia, the area of an individual barrel column (C2 in this illustration) in the PMBSF was mapped based on whisker evoked neuronal responses recorded in layer IV with a microelectrode and using surface blood vessels as bench marks. The barrel column sample was then dissected by aspiration through a micropipette.**(B)** Example of the extent of removed tissue after the dissection of the C2-barrel column. This 2-D coronal view is based on the reconstruction of the serial tangential sections. Horizontal lines indicates the removed part of the barrel cortex. **(C)** Photomicrographs of 6 Nissl-stained tangential sections, corresponding to the reconstruction shown in B. Roman numbers indicate cortical layers. In the sections trough layer IV, some of the barrels that surround the removed C2-barrel are labeled. Scale bars are 500 µm.

collected in this example without encroach upon the neighbouring ones. In figure 11.C., we present 6 photomicrographs from the sections used in the aforementioned reconstruction. The section through layer IV was used to verify the precision by which the C2-barrel column was removed. The lesion was no longer detectable in the section through the subcortical white matter. On the basis of barrel cortex and lesion reconstructions, five samples had to be rejected for biochemical analysis because the

sample included neighbouring barrel columns (not incorporated in the total number of 62 animals described above).

Sample size and protein content. Table 1 presents the parameters that characterize the dissected barrel columns. The radial extent of the extracted column was calculated from the reconstruction of the serial tangential sections. On the basis of these measurements, the volumes of the collected cortical samples were estimated at ~ 0.05 mm^3. In a preliminary experiment we determined the total protein content in 10 extracted barrel columns using Bradford protein assays (BIO-rad, United Kingdom). We found that each barrel column contained 10-15 µg proteins. Given that GLAST and GLT-1 represent as much as 0.2 and 0.8 % of the total protein content in cortical tissue (Lehre and Danbolt, 1998b), the amounts of total protein loaded (~30 µg) in the electrophoresis gels were sufficient to measure relative levels of glutamate transporters using western blots.

Table 1. Barrel column dissection parameters

Parameters	Estimation
Diameter of pipet (in):	220 - 280 µm
Depth of penetration:	800-1000 µm
Presence of lesions until :	800-1000 µm
Volume of samples:	~0.05 mm cube
Quantity of (per sample)	
cortical tissue:	~60 µg
Proteins:	~10-15 µg

Western blots and quantification method

Cortical tissue (2-3 barrel columns pooled) were re-suspended and immediately homogenized in Buffer A (0.32 M sucrose, 10 mM HEPES/KOH pH 7.4, 10 mM DTT) that contains the protease inhibitors PMSF (0.3 mM), aprotinin (2 mg/ml), leupeptin (2mg/ml) and pepstatin (0.7mg/ml). Denaturation was made by boiling for 10 min in an equal volume of Buffer B (2% SDS, 60 mM Tris pH 6.8, 10% glycerol, 5% 2-mercaptoethanol). Electrophoresis of the total proteins was performed on a SDS-PAGE gel. The proteins were then transferred to Protran BA 83 nitrocellulose membrane (Schleicher and Schuel, Germany). Membranes were incubated for 1 hour with blocking buffer (5% milk powder, 1% Tween-20, 150 mM Nacl, 50 mM Tris pH 8.0) and then probed at 4°C overnight with the following primary antibodies: monoclonal anti-actin (1:10.000; Roche, Switzerland), polyclonal anti-GLT-1 (0.05 mg/ml) (Yamada et al., 1998). Membranes were washed and incubated with peroxydase-conjugated secondary antibodies. Chemoluminecsent bands were detected

using a kit (RPN 2106, Amersham, Buckinghamshire, England) and Kodak X-OMAT films. The membrane was then stripped by incubation at 60°C during 1 hour with 10 mM 2-mercaptoethanol, 20% SDS, 1 M Tris-HCl pH 6.8. The membrane was again blocked for 1 hour and probed with a polyclonal anti-GLAST antibody (0.05 mg/ml) (Shibata et al., 1997) or a monoclonal anti-glutamine synthetase antibody (1:500, Interchim, France) and processed as above. Immunoreactive bands on films were digitized and densitometrically analyzed using the NIH Image software. Values of optical densities of the bands for GLAST, GLT-1 and GS were normalized to the optical density of actin in each animal. Differences in the GLAST, GLT-1 or GS relative protein levels between groups were tested using Tukey's studentized range test.

All animal handling procedures used for this study had been approved by the Office Veterinaire Cantonal (Lausanne), in accordance with Swiss Federal Laws

RESULTS

PART I. A 24h period of chronic whisker stimulation induces cortical plasticity in the adult mouse

I.1. Spatiotemporal distribution of evoked current flows in the "stimulated" barrel column

Local field potentials (LFPs) were recorded in the C2-barrel column of animals that were either non-stimulated (NO STIM; n=7) or of those that had been stimulated 24h for their C2-whisker (24h STIM; n=7).

The large variations in evoked field potential as recorded in the extracellular medium of cortical regions are attributable to volume conduction from distant current generators called current sinks and sources. Current source and sinks are commonly thought to be generated mainly by postsynaptic potentials (Mitzdorf, 1985; Leung, 1990). The current source-density (CSD) analysis of extracellular potentials allows the study of the spatiotemporal pattern of synaptic activation evoked by PW-deflection within the barrel column. Spatially, note that these analyses were restricted to one dimension (the z-axe).

Laminar profiles of PW evoked local field potentials. Figure 3.A. shows a representative example of the laminar traces of the field potentials evoked by the deflection of the PW in the C2-barrel column of a non-stimulated mouse. Between mice, the amplitude of evoked potentials varied but the temporal pattern of events was remarkably constant. PW-deflections evoked multiphase local field activity in almost the entire barrel column; the variations in LFP profiles across lamina indicate a heterogeneous pattern of current flows. During the first 40-50 ms of the cortical response, a biphasic extracellular field potential was evoked from 200 μm to 800 μm subpial depth that consisted of a short latency negative potential phase (5-7 ms onset in both groups) immediately followed by a positive phase. In all cases, the relative amplitude of voltage variations were largest between lower layer III and upper layer V and strongly decay with distance outside of this zone. The negative and positive peak amplitudes were always observed in layer IV (400-450 μm depth in both groups). The negative potential phase peaked at 17.1±1.8 ms and 15.7±1.5 ms in NO STIM and 24h STIM groups (mean ± SD). The duration of the initial negative potential phase was

strictly constant across animals and groups (from onset to 25.0±4.1 ms and 24.2±3.4 ms poststimulus in layer IV of NO STIM and 24h STIM groups, mean ± SD). In both the control and the stimulated mice, there were two cases in which the negative-positive potential sequence of the LFP profiles expanded in the upper part of the cortical column (100 μm subpial depth). In the most superficial (0-100 μm depth) and deeper lamina (850-1000 μm depth), field potentials often reversed polarity, with positive-negative phases that were variable in duration across mice.

During the next 50 ms, a biphasic positive-negative potential sequence of small amplitude spatially restricted between lower layer III and upper layer V was observed in both groups. After the late negative phase, the average field potentials gradually returned to the baseline level, which was reach at ~ 100 ms post-stimulus. The total duration of the evoked LFP response was therefore 90-95 ms. Those spatial and temporal patterns of LFPs evoked in a barrel column by the deflection of the PW were not altered by the chronic stimulation.

Spatial and temporal distribution of current sinks and sources. LFPs have poor spatial resolution, therefore voltage traces were processed through one dimensional-CSD analyses to reveal the spatiotemporal distribution of the current sinks and sources at their origin (Nicholson and Freeman, 1975; Mitzdorf, 1985). The CSD traces computed from the laminar LFP traces presented in figure 3.A point up a short latency current sink (positive CSD phase) evoked from layer II to V by the PW-deflection and followed by a current source (negative CSD phase). These currents are more easily identifiable in figure 3.B. that shows two examples of color-coded contour plots, interpolated from the laminar CSD traces recorded in a non-stimulated (left) and in a stimulated mouse (right). These plots illustrate the current sinks (warm colours) and sources (cold colours) variations in function of cortical depth and time.

In both the stimulated and non-stimulated mice, the short latency current sink evoked by the deflection of the PW in layer IV and upper layer V at 5-7 ms poststimulus onset invaded layers II&III within 1-3 ms later. This early current sink peaked in layer IV at a depth of 400-450 μm and had ~ 20 ms duration in both groups. The early sink was balanced by a current source located in layer VI and a smaller one in superficial layer; the latter was more visible in CSD traces than in contour plots. A current source (25 ms poststimulus onset) followed the early sink in layer IV and II&III, lasting until 50 ms poststimulus. Five-six milliseconds after the onset of the early layer IV sink, a current sink progressively invaded infragranular layers and peak 34-45 ms after the stimulus in the middle of layer VI, i.e. concomitant with the peak of the layer IV source. These spatial and temporal distributions of sinks and sources evoked by the deflection of the PW in the barrel column were highly reproducible between mice.

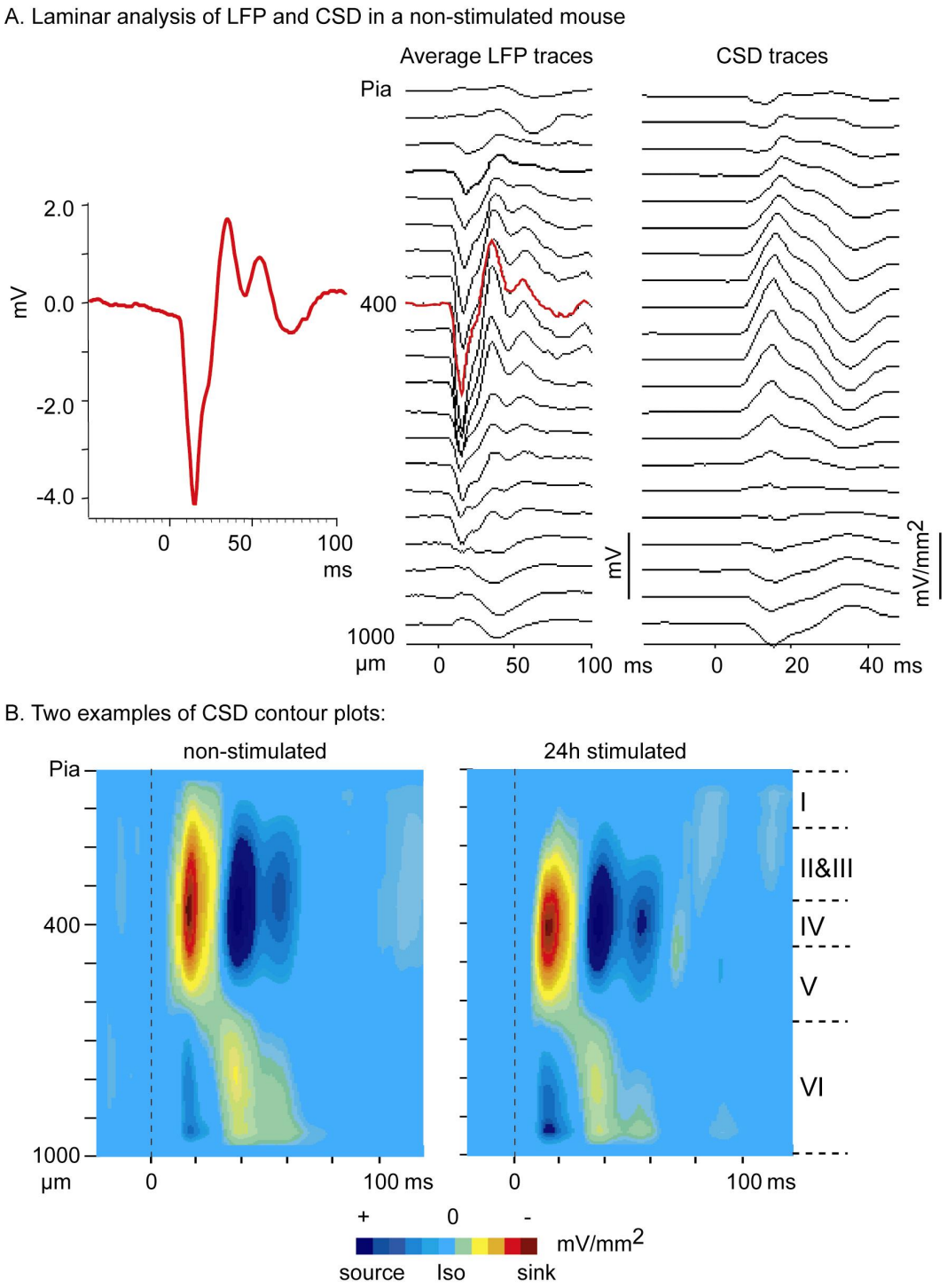


Figure 3. Laminar profiles of local field potentials (LFP) and output of current source-density (CSD) analyses in barrel column C2 upon principal whisker (PW) deflection.

(A) Average traces of the LFPs (left) evoked by 50 successive PW-deflections recorded every 50 µm along the depth of barrel column C2 in a non-stimulated mouse together with the corresponding CSD traces (right). Left inset graph focus on the typical multiphasic LFP evoked in layer IV, with an early negative phase followed by a positive phase, then a positive/negative phase sequence of smaller magnitude. **(B)** Two representatives examples of CSD contour plots computed from the laminar CSD profiles evoked by PW-deflections in the vertical axes of the barrel column C2 from one non-stimulated (same as in A) and of one stimulated mouse. Colour coding shows current sources in blue, current sinks in red and isopotentials in green.

These CSD analyses showed that the initial negative phase of the cortical LFP was the expression of a large current sink. The strong short latency current sink and the corresponding negative potentials evoked by the deflection of the PW correlate with the predominant thalamocortical inputs. The main component of evoked neuronal firing in layer IV temporally matched the initial negative going phase of the LFP and the early current sink (see section I.2.4). No differences were observed between 24h STIM and NO STIM animals in terms of neither laminar location and extent, nor timing of current sinks and sources. Thus, chronic stimulation did not alter the distribution of the current sinks and sources evoked by the PW in the stimulated barrel column.

I.2. Altered neuronal activity in the barrel cortex: quantitative analyses of single units spike discharges

We then analysed the effects of a 24h period of chronic C2-whisker stimulation onto the response properties of individual neurons in the C2- barrel column, as well as in neighbouring columns of barrels C1 and D2 (see figure 1). A total of 902 neurons were recorded in 58 animals from the NO STIM, 52 from the 24h STIM and 18 from the 4d-after-STIM groups. In the stimulated animals, the C2-barrel column will be referred as the "stimulated" barrel column as compared to neighbouring "non-stimulated" barrel columns.

Spiking activity of neurons recorded in layer IV and in layers II&III were analysed separately. In general, neurons recorded in a particular barrel column responded stronger, in terms of the number of spikes evoked, and faster to the deflections of the PW than of the SWs, in agreement with the previous study of Welker et al (1993) in the same mouse strain. These typical response properties of barrel hollow neurons in the PMBSF are illustrated in Figure 4 that shows the AP firing evoked in one layer IV cell encounter in the C2-barrel of a non-stimulated mouse. PSTHs calculated upon 50 deflections of its PW C2 and SWs C1, C3, B1, D2 and D3 are presented. In figure 4.A. and 4.C., the corresponding raster plots towards the deflection of whiskers C2 and C1 are also shown. Typically, this neuron responded faster and fired more spikes upon the deflection of the PW than upon any of the SWs, which reflects the dominance of the PW in the receptive field of barrel neurons. In addition, this example shows that responses to the deflection of whiskers in the surround receptive field vary in function of their position on the whiskerpad: usually in this study the whisker positioned just caudally to the PW within the same row, referred as the in-row caudal whisker (RC; whisker C1 in this case), elicited the strongest response.

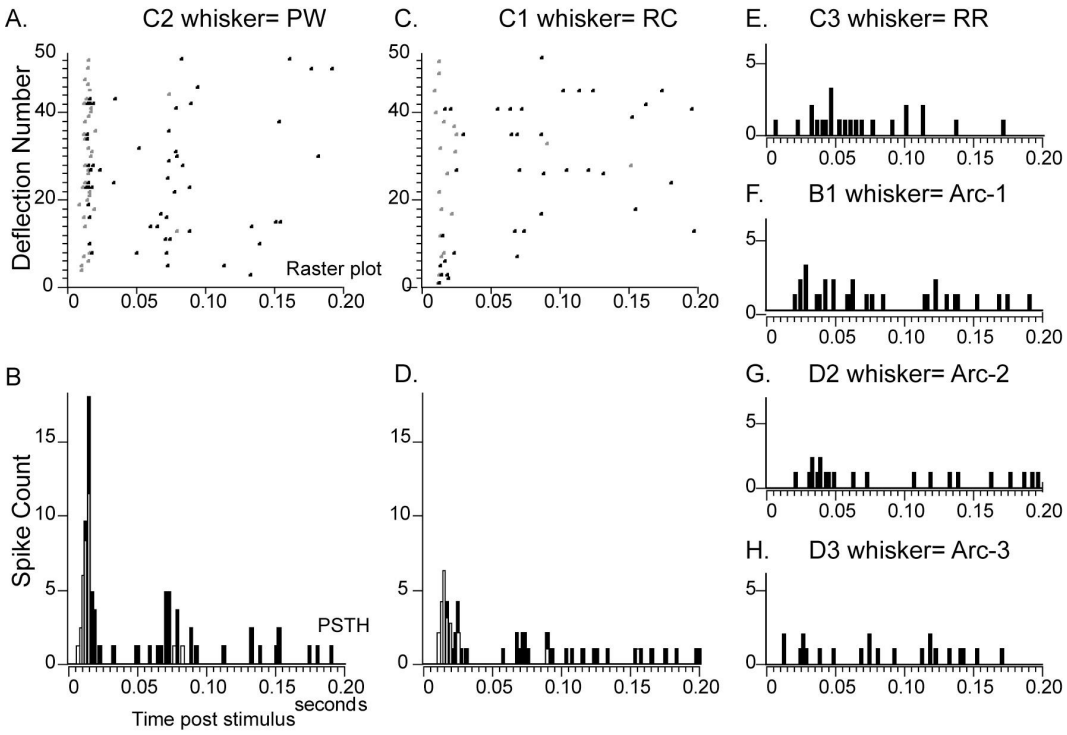


Figure 4. Examples of spiking activity of a layer IV neuron in the C2-barrel towards the deflection of the principal whisker (PW) and 5 surround whiskers (SWs).

(A-D) Raster displays and corresponding PSTHs of spikes elicited in response to 50 successive deflections applied at 0.5 Hz of the C2-whisker (PW) and of the C1-whisker, i.e. the direct caudal SW of the same row (RC). The time scales drawn beneath PSTHs, from the deflection onset to 200 ms poststimulus, apply also for raster displays. White dots in raster plots and white bars in PSTHs represent only the first spike of each response, on which were based LH constructions. **(E-H)** PSTHs of the same neuron towards the deflections of other SWs: in-row whisker C3, rostral to C2 (RR); whisker B1, D2 and D3, whiskers of Arc-1, Arc-2 and Arc-3.

I.2.1. Decreased response magnitude towards the stimulated whisker in the corresponding barrel column

Altered distribution of PW-evoked response magnitude in the stimulated barrel column. Figure 5.A. shows the distributions of neurons encountered in barrel column C2 of the three experimental groups of mice in function of classes of response magnitude values towards deflections of the PW (classes definition: 0.2 spike per deflection). All neurons recorded had significant responses towards the deflection of the PW (>0.1 spike per deflection, above spontaneous activity).

Layer IV. In layer IV of non-stimulated mice, the distribution was centred on the 0.8-1.0 spikes per deflection class; less than 25% of neurons showed responses inferior to 0.6 spikes per deflection. After a 24h period of stimulation, the distribution of layer IV neurons in response magnitude classes was altered as compared to neurons in the non-stimulated animals ($p<0.01$; Chi-square). This distribution became clearly asymmetrical, with 50% of the neurons showing responses inferior to 0.6

spikes/deflection. We observed an increase in the number of neurons that exhibit low response magnitude upon PW-deflection and a decrease of highly responsive units after the chronic stimulation period. Those alterations in the distribution of neuronal responses were transient; on the contrary, we observed a tendency towards an increase in the number of highly responsive neurons (>1.4 spikes/deflection) in the 4d-after-STIM group of mice as compared to NO STIM mice.

Layers II&III. In layers II&III, the distribution of neurons in the classes of response magnitude was shifted towards smaller values after the 24h period of stimulation ($p<0.01$); 4 days after the stimulation was halted, the distribution of cells in function of their response magnitude was no longer significantly different than in non-stimulated animals.

Transient decrease in mean response magnitude upon PW-deflections in the stimulated barrel column. In correspondence with the shift in RM distribution, the mean response magnitude of neurons upon PW-deflection in the 24h STIM group was significantly smaller in both layer IV and layers II&III as compared to the NO STIM and 4d-after-STIM groups ($p<0.05$; MANOVA and Tukey's HSD tests). Mean response magnitudes in the C2-barrel column towards the PW-deflection in the three experimental groups are shown in figure 5.B.

Layer IV. After a 24h period of whisker stimulation, mean response magnitude of neurons to the deflection of the PW had decreased by 25% as compared to non-stimulated mice (mean ± SD= 0.97±0.53, n=102 in NO STIM vs. 0.71±0.40, n=77 in 24h STIM; $p<0.01$). This decrease was transient as in mice analysed 4 days after the stimulation period, mean response magnitude upon deflection of the PW was even increased as compared to non-stimulated mice by 15 % (4d-after-STIM: 1.14±0.52, n=82; $p=0.03$).

Layers II&III. The depressive effect of the chronic whisker stimulation onto neuronal responses in the corresponding barrel column tended to be stronger in layers II&III than in layer IV, as mean response magnitude upon deflection of their PW for neurons in the group of chronically stimulated mice was reduced by 36% as compared to mean response magnitude in non-stimulated mice (mean ± SD= 0.74±0.36, n=73 in NO STIM; 0.47±0.36, n=70 in 24h STIM; $p<0.01$). Four days after, mean response magnitude of neurons was not significantly different from neurons in non-stimulated mice (4d-after-STIM: 0.67±0.32, n=53).

Response magnitude upon PW-deflection in adjacent non stimulated barrel columns. Mean response magnitude for neurons in layers II&III and in layer IV of barrel columns C1 and D2 towards the deflection of their own PWs were compared between 24h chronically stimulated and non-stimulated mice (see figure 5.B). No statistically significant differences in mean response magnitude of neurons were revealed at $p<0.05$, indicating that the decrease in PW-evoked response was restricted to the barrel corresponding to the chronically stimulated whisker, despite somewhat smaller values

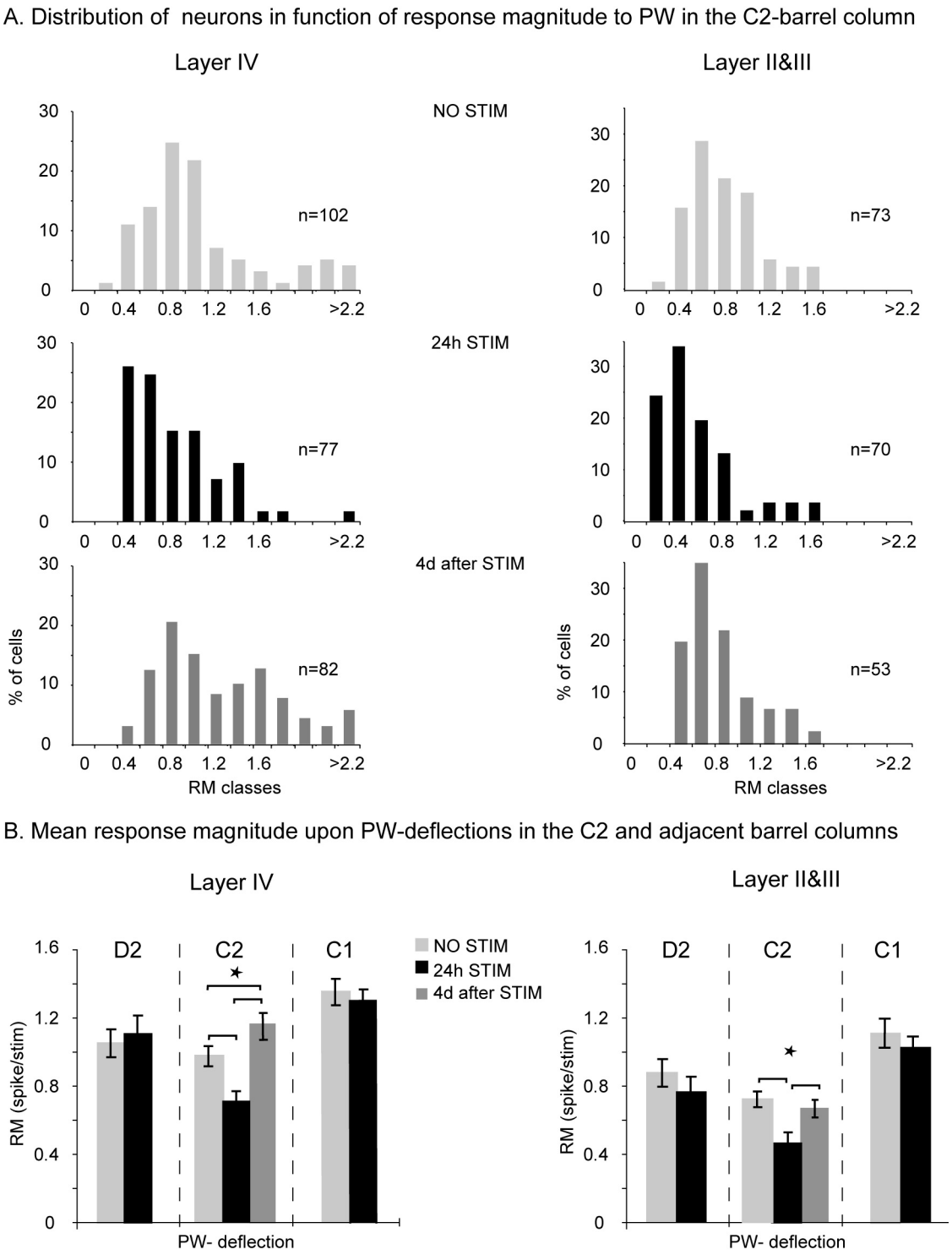


Figure 5. Localized effects of chronic C2 whisker stimulation onto principal whisker (PW) response magnitude (RM) in the barrel cortex.

(A) Distributions of PW-evoked RM for neurons in layer IV and layers II&III of the C2-barrel. Neurons were classified in successive RM classes of 0.2 spikes per deflection (n= numbers of neurons in each sample). In both layer IV and layers II&III, Chi-square analysis revealed significant differences between RM distributions in the 24h STIM group of mice as compared to mice in the NO STIM and in the 4d-after-STIM groups ($p<0.01$). In layer IV, RM distributions were found to be significantly different between the NO STIM and the 4d-after-STIM groups ($p<0.01$). **(B)** Mean response magnitude ± sem for neurons in layer IV and layers II&III of barrel columns C2, C1 and D2 to the deflection of their respective PW. A transient significant decrease in mean principal whisker RM was observed after chronic stimulation in both layers, restricted to the barrel column corresponding to the stimulated whisker (MANOVA and Tukey's HSD; $p<0.01$). In layer IV of the C2-barrel, mean RM to C2-whisker was significantly higher for neurons of the 4d-after-STIM mice as compared to these of non-stimulated mice ($p<0.05$).

of mean response magnitude in layers II&III of both C1 and D2 barrel columns of the 24h STIM group as compared to the NO STIM group.

Thus, after a 24h period of chronic stimulation of the C2-whisker, neurons in the C2-barrel column showed decreased responses towards the deflection of this whisker. This decrease in PW-evoked response was stronger in layers II&III than in layer IV and was restricted to the barrel column corresponding to the stimulated whisker as PW-evoked responses in adjacent barrel columns were unaltered. This depression of PW-evoked responses was transient as mean response magnitude of neurons recorded 4 days after the stimulation period was no longer smaller than in non-stimulated animals. However, a long lasting effect of whisker stimulation persists as mean response magnitude in layer IV of the 4d-after-STIM group of mice was significantly higher than in the NO STIM group.

I.2.2. Decreased spontaneous activity in the stimulated barrel column

Neurons were recorded during the cortical active periods of slow wave sleep and under this condition most of cells were spontaneously active. In non-stimulated mice, the range of spontaneous activity was 0-6 Hz in layer IV with a mean ± SD value of 1.7±0.8 Hz and 0-4 Hz in layers II&III with a mean value of 1.3±0.8 Hz. After a period of 24h whisker stimulation, mean spontaneous firing of neurons was significantly reduced by 47% and 40% in layer IV and layers II&III of the stimulated barrel column (layer IV: 0.9±0.3 Hz; layers II&III: 0.8±0.5 Hz; $p<0.01$, Kruskall-Wallis) as compared to values in non-stimulated animals. In the C2-barrel column of mice of the 4d-after-STIM group, spontaneous activity was similar to values in non-stimulated mice. This transient decrease in spontaneous activity was restricted to barrel column C2 as spontaneous activity in barrel columns C1 and D2 was identical in mice of the 24h STIM group and of the NO STIM group.

I.2.3. Unaltered modal latency of PW-evoked response in the stimulated barrel column

Figure 6 presents for the three experimental groups the mean modal latencies of the responses of neurons in the C2-barrel column towards the deflection of their PW. Modal latency represents the mode of the time occurrence of the first spike recorded after the onset of the whisker deflection. Layer IV neurons responded to the deflection of the PW with a mean ± SD modal latency of 11.4±3.8 ms in non-stimulated mice, 10.9±3.4 ms in the 24h STIM group and 10.7±2.7 ms in mice of the 4d-after-STIM group. In layers II&III, the modal latency of neuronal responses towards the deflection of the PW was respectively 14.7±5.8ms, 14.8±8.8 and 13.9±3.4 ms in the three experimental groups. Statistical analysis confirmed that modal latency of the response to the deflection of the PW was not modified by the chronic stimulation period neither in layer IV and II&III (Tukey's HSD tests).

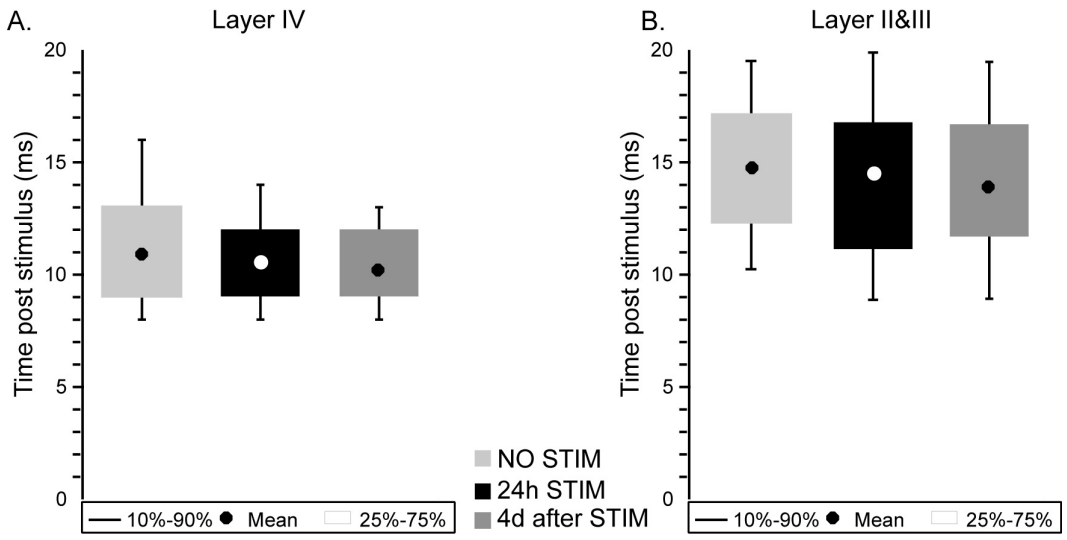


Figure 6. Unaltered modal latency towards the PW in the C2-barrel column.
(A) Mean modal latency of layer IV neurons responses in the C2-barrel towards the deflection of their PW was not significantly modified by chronic whisker stimulation. **(B)** Mean modal latencies of layers II&III neurons responses in the C2-barrel column in the three experimental groups. No significant differences were observed between groups.

I.2.4. Intra-cortical origin for the decreased in PW-evoked response: time epoch analysis

We then performed a time epoch analysis of PSTHs in order to determine whether the decrease in response magnitude of neurons in the C2-barrel column towards the deflection of the PW reflected a general suppression of evoked firing or affected only parts of the cortical response. First, mean population PSTHs towards the PW were calculated using the PSTHs of all neurons in a given layer and experimental group. Mean population PSTHs data were expressed as the mean number of spikes evoked per single whisker deflection within bins of 1 ms, and corrected for spontaneous activity. Results of this analysis are presented in figure 7.A. Second, to allow quantitative measurements and statistical comparisons between groups, the PSTHs of all neurons were subdivided in 5 poststimulus time epochs. These time epochs matched salient periods of the profile of the layer IV neuronal firing evoked by the deflection of the PW in the C2-barrel, as delineated with mean population PSTHs (see below). Mean frequencies of spiking per ms during each epoch were then calculated and averaged across neurons per layers and experimental groups.

Layer IV. The lower mean population PSTH presented in figure 7.A. shows that in all groups, layer IV neurons began to fire action potentials above spontaneous activity as soon as 6 ms after the onset of whisker deflection (a 1 ms time-bin was considered as responsive if spiking probability exceeds 0.0015 above spontaneous activity and that this time-bin was part of at least 3 successive bins firing above spontaneous level). The

peak in spiking probability was reached 11 ms poststimulus, defining the first time epoch (T1:]3-11 ms). Spiking probability then gradually decreased and returned to spontaneous level around 25 ms poststimulus (epoch T2: 12-25 ms). During the next 25 ms, neurons did not have spiking probabilities above spontaneous level (epoch T3: 26-50 ms). Then a rebound of activity took place that last until 100 ms poststimulus with a smooth peak around 70 ms (epoch T4: 51-100 ms), after which spiking probabilities returned to spontaneous level.

The temporal profile of the response time course was conserved after chronic whisker stimulation, indicating that the temporal tuning of spike probability was not altered. Quantification of mean frequency per epoch revealed several striking facts (see figure 7.B.). Mean frequency during the first time epoch was not significantly modified by the chronic stimulation period (29.5±3 Hz in 24h STIM vs. 27.6±3 in NO STIM). During the second component of the response (T2) however, mean PW-evoked spiking was drastically reduced in chronically stimulated animals as compared to non-stimulated and to 4d-after-STIM animals ($p<0.01$, ANOVA repeated measures on mean frequency per epoch). As a consequence, mean spiking frequencies during T2 in 24h STIM animals was smaller than during the initial period T1, which was not the case in the NO STIM and 4d-after-STIM mice. During the third epoch (T3) neuronal activity remains at spontaneous level in all groups. The characteristic rebound in neuronal activity evoked by the deflection of the PW during T4 was strongly reduced in 24h STIM mice relative to non-stimulated mice ($p<0.01$). Four days after however, evoked spiking was significantly enhanced during this late poststimulus time epoch as compared to non-stimulated animals at $p<0.05$ ($p=0.02$).

Layers II&III. The onset of evoked activity in layers II&III began at 6 ms poststimulus, as for layer IV neurons, presumably under monosynaptic activation by thalamo-cortical axons which provide layers II&III neurons with some inputs (see figure 7.A.). Peak of mean evoked spiking probability in the neuronal population of supragranular layers was reached 2-3 ms later than in layer IV, culminating at 13-14 ms poststimulus. Spiking probability then gradually decreased and around 25 ms poststimulus reached a plateau of low probability of spiking above the spontaneous level, until 100 ms poststimulus. When compared to non-stimulated mice, evoked population spiking in layers II&III of stimulated animals of the 24h STIM and 4d-after-STIM groups was of same duration and conserved the same temporal profile.

The same time epochs were used for the analysis of response of neurons in layers II&III as in layer IV. The outcome of this analysis reveals that 24h of chronic whisker stimulation does not alter mean spike frequency during the first epoch ($p=0.7$) but strongly decreased evoked firing during the second time epoch T2, as compared to non-stimulated mice ($p<0.01$; ANOVA with repeated measures). Mean population PSTH histograms show that after the initial epoch T1, during which layer IV neurons reached their maximal firing, spiking probability strongly increased until a peak at 13-14 ms in layers II&III of non-stimulated animals, whereas in stimulated animals mean

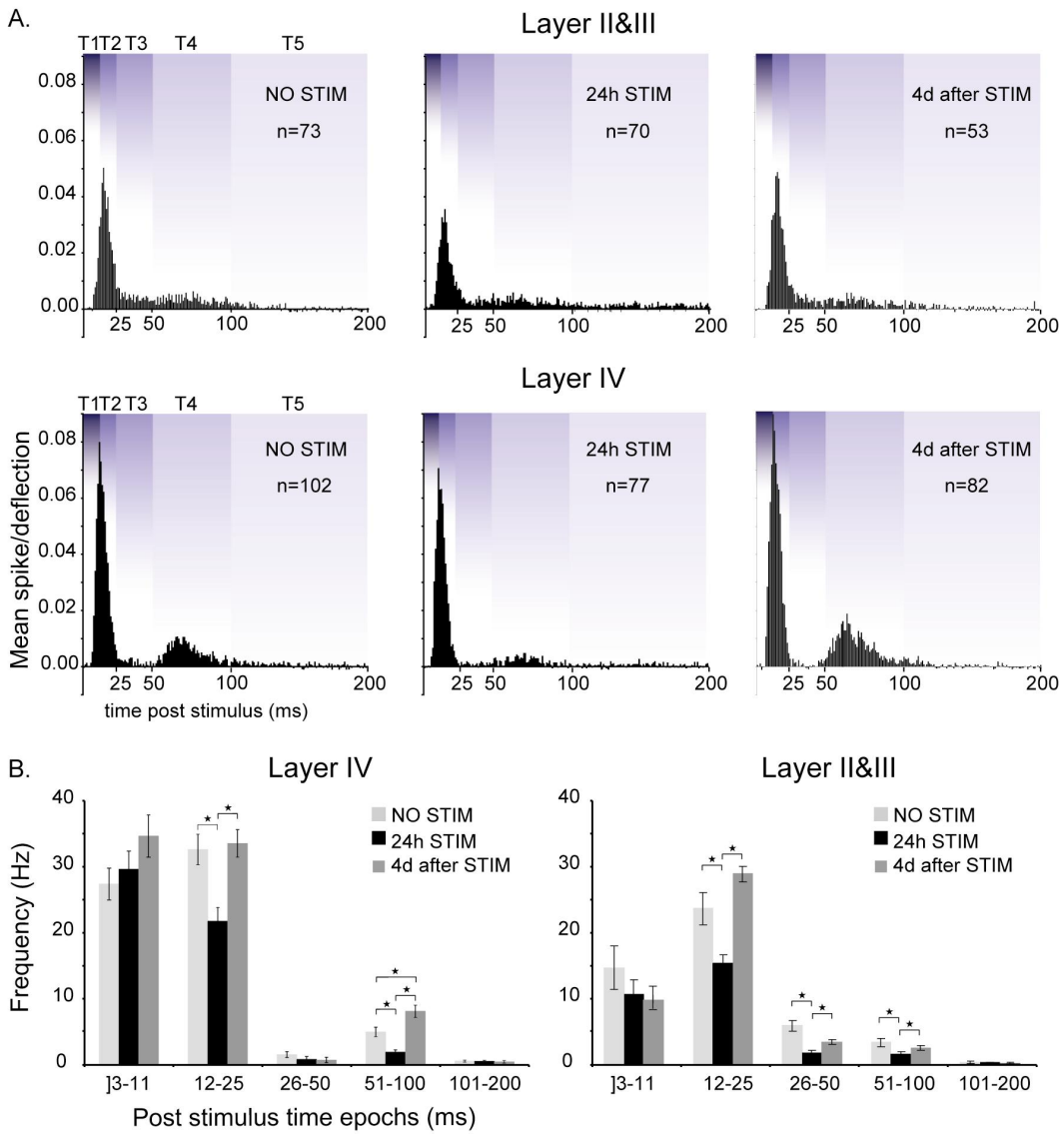


Figure 7. Time epoch analyses point to an intracortical origin for the decrease in PW evoked response after chronic whisker stimulation.

(A) Mean population PSTHs histograms calculated upon C2 whisker deflection in layer IV and layers II&III of the C2-barrel column (mean number of evoked spike per neuron and per deflection within bins of 1 ms). The temporal profile of the mean population PSTHs calculated in layer IV of non-stimulated animals was used to determine 5 successive time epochs. Initial activation epoch ends at the peak in spiking probability (T1= 3-11 ms). Second epoch is characterized by a decreasing firing (T2= 11-25 ms) and followed by an epoch during which activity does not exceeded spontaneous level (T3= 25-50 ms). Fourth epoch was designed to encompass the typical rebound in firing probability (T4= 50-100 ms). During the last epoch, neuronal firing probability returns to spontaneous level (T5= 100-200 ms). Same time epochs were used for layers II&III PSTHs. Time epochs are labelled above the first histogram and drawn behind each graphs by graded colours. **(B)** Mean population frequency per ms and per time epochs. Asterisks indicate significant differences between groups at $p<0.05$ (ANOVA for repeated measures). After chronic stimulation, neuronal response was significantly decreased during T2 and T4 in layer IV, and during T2, T3 and T4 in layers II&III; 4 days after the stimulation period, it returned to non-stimulated level. Spiking frequencies remained unaltered during the earliest epoch corresponding to the initial period of activation of the barrel column. Note that response was significantly higher during the fourth time epoch after a stimulus-free period of 4 days, as compared to the NO STIM group. Mean values are given ± sem.

spiking probability did not exceed the level attained at 11 ms poststimulus, and decreased rapidly after 13 ms poststimulus. Whereas mean spiking frequency was significantly amplified during epoch T2 as compared to epoch T1 in the NO STIM ($p<0.01$), spiking frequency during T2 in 24h STIM animals only moderately exceeded the level sustained during T1. During the following time epochs T3 and T4, spike frequencies were significantly lower in chronically stimulated as in non-stimulated animals ($p<0.01$). Mean frequency per epoch was not significantly different in the 4d-after-STIM and the NO STIM groups at each epoch.

I.2.5. SW-evoked responses in the stimulated barrel column: not all responses are altered by chronic whisker stimulation

Latency analysis in the C2-barrel column. Median latencies and interquartile range of PW- and SW-responses in the C2-barrel column are given in Table 2 for all experimental groups. Median latencies were used here because the calculation of modal latency requires a clear peak in the distribution of first spikes within the LHs which was not always the case for SW-responses. Responses to PW was evoked at faster latency than responses to all SWs at $p<0.01$ in both layer IV and layers II&III and in all groups of mice (Kruskall-Wallis). Across SWs, the deflection of whiskers C1 and C3 evoked faster responses than the deflection of rows B and D whiskers at $p<0.05$ in layer IV neurons. In layers II&III neurons however, the differences between latencies of neurons to the deflection of whiskers C1 and C3 as compared to the other SWs did not systemically reach a significant level. In the majority of cases, latencies upon the deflection of row D whiskers were faster than upon the deflection of row B. Note that while latencies to the PW were faster in layer IV than in layers II&III, no statistically significant differences were observed when the responses towards each SW were compared between layers within each group. Finally, between groups comparison revealed that chronic stimulation did not altered median latencies of any SW responses at $p<0.05$ (MANOVA and Tukey's).

Response magnitude towards SWs in the C2-barrel column. In layer IV and layers II&III of barrel column C2, the response magnitude to the deflection of a SW depends on its position on the whiskerpad relative to the PW. In the C2-barrel, caudal in-row whisker C1 evoked the strongest response in non-stimulated animals followed by the rostral in-row whisker C3 (at $p<0.05$, Kruskall-Wallis). With respect to responses evoked by the deflection of whiskers in rows B and D, we noticed that the caudal whiskers (Arc-1) evoked stronger responses in barrel column C2 than the rostral whisker on the Arc-3; however these differences did not reach significant levels.

Table 2. Median latency and interquartile range (ms). Barrel column C2

Barrel	Groupe	Whisker	Layer IV			Layer II&III		
			Percent responsive	Median	IQ	Percent responsive	Median	IQ
C2	NO STIM	PW: C2	100 (n=102)	13.0	4.0	100 (n=73)	16.5	7.0
		C1	76	22.8	23.3	79	26.5	21.6
		C3	63	27.0	27.0	70	30.0	18.3
		D1	55	40.0	27.8	56	30.0	26.4
		D2	62	37.0	22.5	66	26.0	18.0
		D3	50	48.0	29.4	39	32.8	22.8
		B1	58	55.5	24.0	58	46.0	20.0
		B2	58	48.0	28.8	65	51.3	25.4
		B3	49	47.5	18.6	34	56.0	13.5
	24h STIM	PW: C2	100 (n=77)	12.0	3.0	100 (n=70)	15.0	9.0
		C1	46	23.0	21.8	47	32.0	26.5
		C3	48	27.0	30.3	59	35.5	25.0
		D1	54	52.0	38.0	52	44.0	19.0
		D2	55	36.0	36.3	57	37.0	24.5
		D3	45	39.0	35.0	38	46.0	21.3
		B1	53	48.5	38.0	45	51.0	23.0
		B2	46	53.0	26.0	61	50.5	27.8
		B3	47	58.0	23.0	40	53.5	19.0
	4d after STIM	PW: C2	100 (n=82)	13.0	3.0	100 (n=53)	17.0	5.3
		C1	68	27.5	32.0	82	31.8	17.9
		C3	63	25.0	29.6	63	36.8	13.8
		D1	48	57.0	19.0	76	42.0	18.5
		D2	61	38.5	32.3	55	30.5	11.0
		D3	49	43.0	25.6	39	55.5	17.0
		B1	56	50.5	17.3	43	53.5	20.0
		B2	57	46.0	24.0	55	40.5	25.5
		B3	44	58.0	17.1	38	56.5	26.3

Therefore, responses to SW deflections were grouped as follows (see upper diagram in figure 8): responses to caudal whisker C1 and rostral whisker C3 were kept separated, responses to whiskers from Arc-1 (B1, D1) were pooled, as well as responses to Arc-2 (B2, D2) and Arc-3 (B3, C3) whiskers. Two further observations are worth mentioning. (1) The number of SWs eliciting activity above spontaneous level per neuron was not altered, suggesting that the size of the surround receptive field was not diminished by the stimulation (see Table 2; percent responsive columns). In layer IV and layers II&III, less than 10 % of neurons only responded to the PW and most surround receptive field comprised up to 5 whiskers. In layer IV, 5% of neurons responded to the 8 adjacent SWs and up to 10% of layers II&III neurons exhibit such large receptive fields. (2) The PW-deflection invariably evoked strongest response than any of the SWs for all neurons in layer IV and the vast majority of neurons in layers II&III for the three experimental groups. The strict respect of this PW dominance in our recordings was presumably due to the fact that the barrel septa were

avoided during this study and that electrode penetrations were made perpendicular to the pial surface. Figure 8 presents the mean response magnitude of neurons in the three experimental groups upon the deflection of the PW and all SWs.

Layer IV. For comparisons between groups, response magnitude values of neurons towards the deflection of individual whiskers were processed as dependant variables. The MANOVA test revealed a significant group effect in layer IV at $p<0.05$. Multiple comparisons were then processed for each individual whisker evoked responses and across the three experimental groups using Tukey's test. It showed that not only the response to the PW was depressed following chronic stimulation: in the 24h STIM group, response magnitude towards the deflection of the caudal SW C1 was significantly lower by 42 % as compared to the NO STIM group (Mean ± SD: 0.29±0.25 in No STIM vs. 0.17±0.23 in 24h stim.; $p<0.01$; figure 8). Response magnitude to rostral SW C3 was also reduced after stimulation, however this decrease did not reach significant level. Responses evoked by SWs on Arc-1 to 3 were not affected. Four days after the stimulation was stopped, a tendency towards lower values of mean response magnitude were still observed towards the deflection of the C1- and C3-whiskers compared to non-stimulated mice, but these differences were not statistically significant.

Since the C1-evoked response was modified in the stimulated barrel, I included the corresponding mean population PSTHs for the three groups of mice in figure 8. The first time bin bearing evoked activity above spontaneous firing occurs at 9-10 ms poststimulus, the peak of activity was reached around 17-18 ms poststimulus then firing gradually decreased until a long lasting plateau of low firing took place. The temporal response profile remained unaltered after the 24h period of chronic stimulation and modal latency of neurons towards the deflection of SW C1 was not altered by chronic stimulation (20.7±17.5 ms in NO STIM, 19.3±18.2 ms in 24h STIM, 24.0±14.4 ms in 4d-after-STIM; mean ± SD). However, evoked spiking probability was clearly lowered during almost the entire duration of the response, i.e. from T2 to T4, in the 24h STIM group of animal as compared to the NO STIM and the 4d-after-STIM groups.

Layers II&III. The MANOVA test revealed a significant group effect onto response magnitude of neurons in layers II&III of the stimulated barrel at $p<0.05$. Chronic sensory stimulation led to similar effects as in layer IV: besides a decrease in PW-evoked mean RM, we observed a 52% decrease in mean response magnitude towards the deflection of the C1-whisker (0.32±0.30 in No STIM vs. 0.15±0.23 in 24h stim.; $p<0.01$; figure 8). Mean response magnitudes towards the deflection of all the other SWs were not significantly different between groups. Figure 8. presents the mean populations PSTHs of neurons towards the deflection of SW C1. Onset of evoked

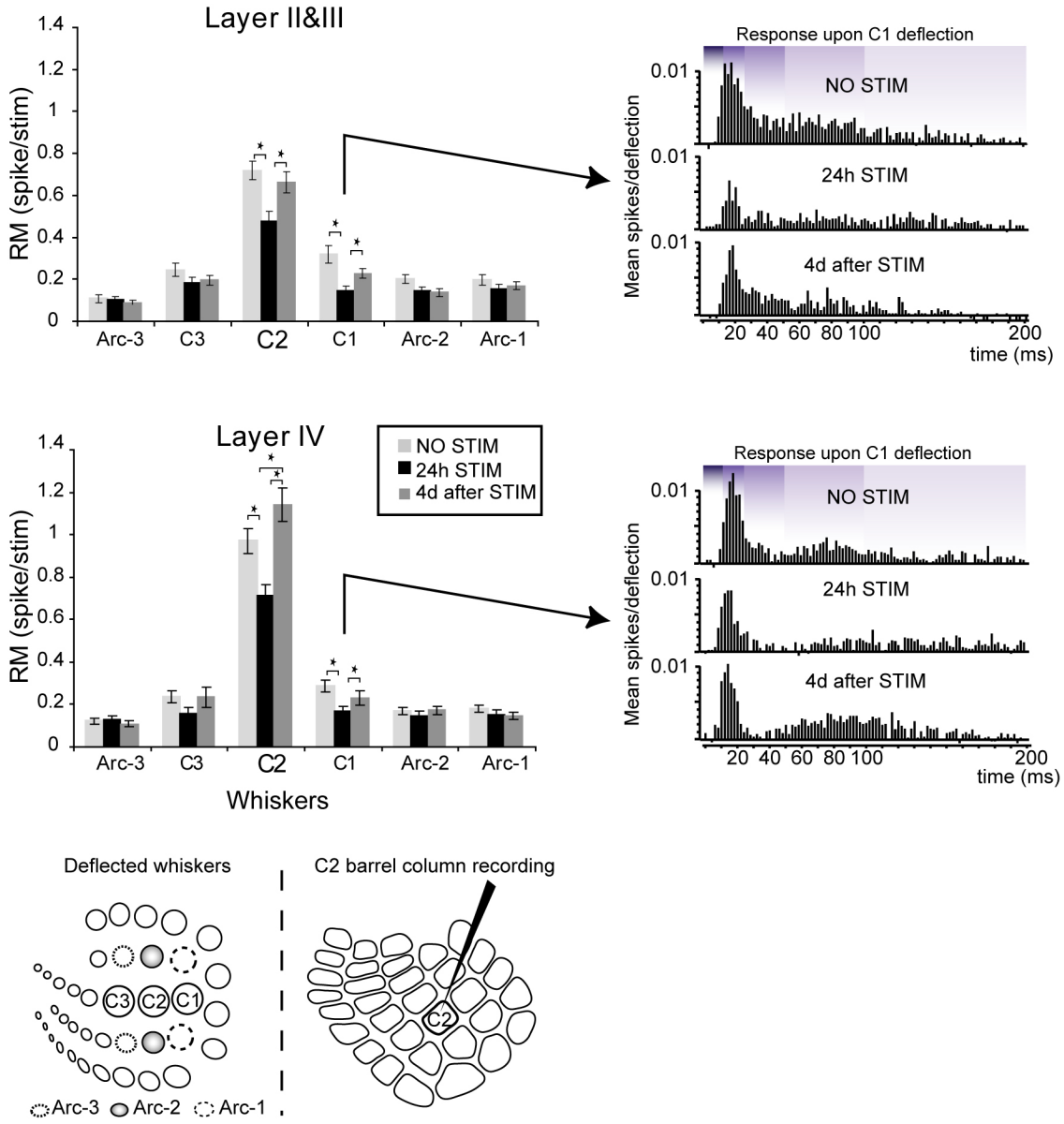


Figure 8. Modifications in principal versus surround whisker response magnitude (RM) in barrel column C2 after chronic whisker stimulation.

Mean RM ± sem for layer IV and layers II&III neurons upon PW-and SWs-deflections. Responses towards SWs that did not belong to row C were pooled in 3 sets (see lower diagram): Arc-1 (B1+D1), Arc-2 (B2+D2) and Arc-3 (B3+D3). Asterisks indicate values that differ significantly between groups at $p<0.05$ (Hierarchical multivariate ANOVA). RM towards the PW decreases after chronic stimulation (same datas as in Figure 5). RM towards SW C1 also robustly decreases in layer IV and layers II&III. Responses to whisker C3 were smaller but not significantly different. Responses to other SWs were not altered after whisker stimulation. Mean population PSTHs (2 ms bins) towards the C1-whisker are given next to RM histograms. Almost the overall profile of spiking probability towards whisker C1 was lowered after a 24h period of C2 whisker stimulation as compared to non-stimulated mice and to mice that were recorded 4 days after the cessation of the stimulation.

activity above spontaneous level occurred at 9-10 ms poststimulus, rapidly reached maximal firing probabilities, then decreased to a long lasting plateau of low spiking probability around 25 ms poststimulus in non-stimulated mice. After stimulation, a drastic reduction in firing affected the entire duration of the response. Modal latency to whisker C1-deflection was not altered by chronic stimulation (23.3±15.3 ms in NO STIM, 19.7±7.8 ms in 24h STIM, 23.3±15.0 ms in 4d-after-STIM; mean ± SD).

I.2.6. Layer specific reduction of the response to the stimulated whisker in "non-stimulated" barrel columns

Evoked responses of neurons towards the deflection of their SWs, including whisker C2, were also recorded in the C1- and D2-barrel columns for the NO STIM and 24h STIM groups.

General observations. The data sets for both barrel columns were analyzed according to a multivariate hierarchical statistic procedure which considered the responses of neurons to all whiskers (PW + SWs), classified by layers, as dependent variables. The MANOVA test did not detect a global group effect of the chronic stimulation onto response magnitude neither in the C1- nor in the D2-barrel columns (barrel C1: p=0.83 and 0.28, barrel D2: p=0.84 and 0.34; RM in layers IV and II&III respectively). However, this model could have underestimated the potential effect of the stimulation period onto response magnitude of neurons towards individual whiskers as it was designed to be highly robust, minimizing type II error (false positive). Therefore, we decided to compare separately mean response magnitude of neurons to each individual whisker deflection between the two groups of animals using ANOVA or non parametric statistics, despite the MANOVA test did not identify overall differences at a significant level.

Layer IV. No significant modifications of response magnitude were observed at $p<0.05$ upon any deflected whisker in the 24h STIM group as compared to the NO STIM group, neither in barrel C1 or D2 and despite a tendency towards lower mean response magnitudes in the C1-barrel (figure 9.A and 9.B). Table 3 presents median latencies and IQ range values of responses in layer IV and layers II&III of the C1- and D2-barrel column towards the deflection of all the whiskers tested for the NO STIM and 24 STIM groups. In both groups, the C2-whisker evoked faster responses in the C1-barrel than in the D2-barrel (C1-barrel= 24.0±28.0 ms and 21.5±6.9 ms, barrel D2= 43.0±22.4 ms and 42.3± 24.0 ms; Median latency ± IQ). In conclusion, chronic stimulation did not alter the latencies of neuronal responses.

Layers II&III. Mean response magnitude towards the deflection of the C2-whisker in layers II&III was significantly decreased in both barrel C1 and D2 of the 24h STIM group as compared to the NO STIM group at highly robust p values (C1-column: p= 0.007; D2-column: p= 0.0038; Kruskall-Wallis). Mean population PSTHs for the deflection of the C2-whisker in layers II&III of both the C1- and D2-barrel columns (figure 9.A. and 9.B.) show that evoked activity was decreased during the entire

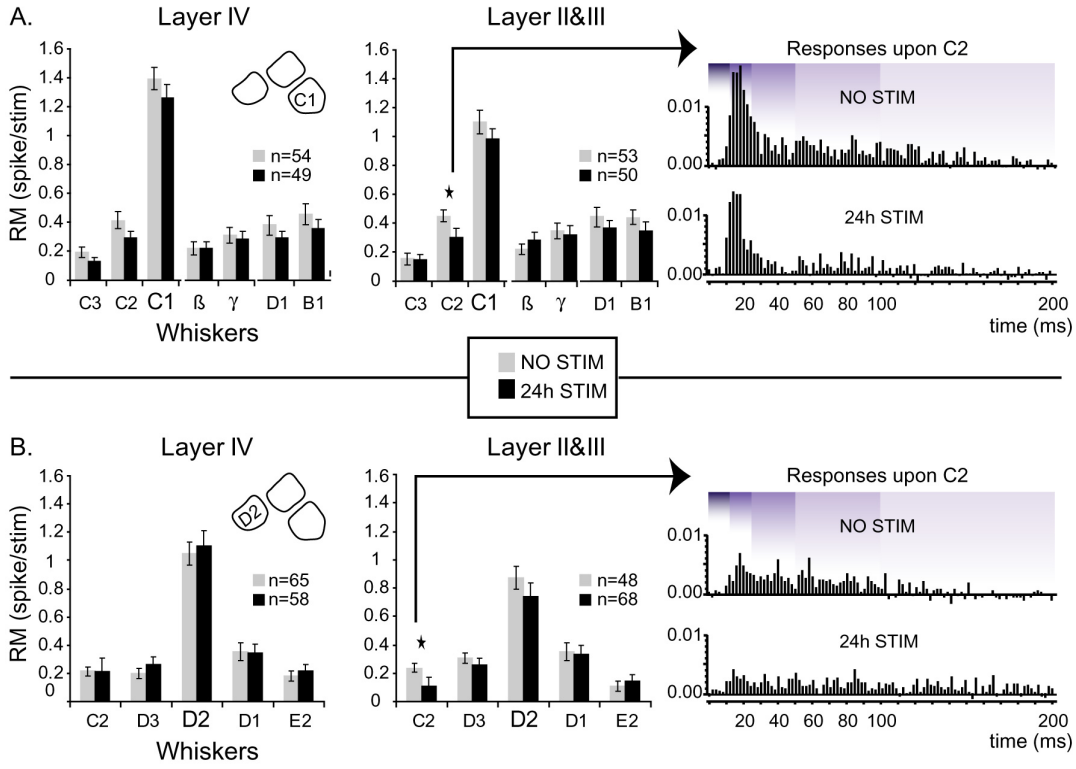


Figure 9. Effects of chronic C2-whisker stimulation onto response magnitude (RM) in barrel columns C1 and D2 upon deflection of their PW and SWs.

(A) Mean RM ± sem of neurons in layer IV and layers II&III of barrel column C1 upon deflections of the PW and 6 SWs (NO STIM: N=54 and 53 in layer IV and layers II&III. 24h STIM: n=49 and 50 in layer IV and layers II&III). Mean RM of neurons in layers II&III but not in layer IV was significantly decreased upon deflection of the stimulated C2-whisker. RM upon deflection of the PW and the other SWs were not significantly modified. **(B)** Mean RM ± sem for neurons in barrel column D2 upon deflections of the PW and 4 SWs. (NO STIM: N=65 and 48 in layer IV and layers II&III. 24h STIM: n=58 and 68 in layer IV and layers II&III). Responses of layer IV neurons were not affected by the chronic stimulation in barrel D2. In layers II&III, C2-whisker evoked RM was decreased after chronic stimulation whereas responses towards the deflection of all other whiskers were not altered. Asterisks indicate values that differ significantly between groups at $p<0.01$ (Kruskall-Wallis). Mean population PSTHs (2 ms bins) of layers II&III neurons towards the C2-whisker are presented next to RM histograms.

duration of the response. Mean response magnitude of neurons towards the deflection of their respective PWs and all other SWs were not significantly modified by the chronic whisker stimulation period at $p<0.05$. The specific decrease upon the deflection of the C2-whisker solely and the deliberate robustness of our multivariate model could explain why the MANOVA test did not reveal an overall group effect. As in layer IV, mean response magnitude to whisker C2 deflection was higher in barrel column C1 than in D2, and latencies tended to be faster in barrel column C1 than in D2. (C1-column= 28.5±22 ms and 21.0±10.0 ms, barrel D2= 33.8±24.0 ms and 40.5±24.5 ms, in NO STIM and 24h STIM respectively; Median latency ± IQ). Also in layers II&III, chronic stimulation did not alter the latencies of neuronal responses.

In summary, the chronic stimulation of a whisker did not affect neuronal activity beyond the corresponding barrel column, i.e. the stimulated barrel column, in terms of spontaneous activity and PW-evoked and SW-evoked responses, except for the responses evoked by the deflection of the chronically stimulated whisker which were significantly decreased at the level of layers II&III in non-stimulated barrel columns.

Table 3. Median latency and interquartile range (ms). Barrel columns D2 and C1

Barrel	Groupe	Whisker	Layer IV			Layer II&III		
			Percent responsive	Median	IQ	Percent responsive	Median	IQ
D2	NO STIM	PW: D2	100 (n=65)	13.0	3.0	100 (n=48)	17.0	5.0
		D1	73	35.5	36.5	79	28.0	25.0
		D3	63	28.5	28.8	62	24.5	12.5
		C1	56	46.3	17.5	46	44.5	10.5
		C2	69	43.0	22.4	76	33.8	24.0
		C3	50	47.5	11.8	44	55.5	19.3
		E1	38	41.5	20.3	58	39.5	25.3
		E2	65	37.0	23.0	33	37.0	16.3
		E3	34	54.3	24.8	30	32.0	8.6
	24h STIM	PW: D2	100 (n=58)	13.0	3.3	100 (n=68)	16.0	6.0
		D1	77	30.5	20.0	85	29.0	23.5
		D3	60	26.0	8.0	71	29.5	16.5
		C1	58	45.5	16.0	71	52.8	25.8
		C2	50	42.3	24.0	47	40.5	24.5
		C3	51	50.5	16.5	32	37.5	20.8
		E1	42	40.5	31.4	48	37.0	26.0
		E2	60	44.0	30.5	48	40.0	25.5
		E3	36	37.0	21.5	43	37.0	18.0
C1	NO STIM	PW: C1	100 (n=54)	12.0	3.0	100 (n=53)	15.8	6.0
		C2	83	24.0	28.0	92	28.5	22.0
		C3	59	38.0	30.0	57	34.7	17.2
		β	58	50.5	20.0	64	45.0	19.3
		γ	72	39.0	26.8	71	34.3	26.4
		B1	88	44.0	26.6	86	43.3	19.5
		B2	38	55.0	16.0	45	42.0	29.4
		D1	78	42.0	28.5	76	28.5	18.3
		D2	40	41.0	26.0	39	26.5	17.0
	24h STIM	PW: C1	100 (n=49)	12.0	3.0	100 (n=50)	16.0	6.5
		C2	70	21.5	6.9	61	21.0	10.0
		C3	52	44.0	15.0	42	40.5	23.2
		β	63	44.0	18.5	66	36.0	26.5
		γ	63	30.0	32.0	68	41.0	24.8
		B1	79	41.8	28.3	70	34.3	26.0
		B2	37	49.0	10.5	44	57.3	21.8
		D1	77	40.5	24.4	71	43.3	14.0
		D2	41	52.0	23.8	41	51.0	12.0

I.3. Chronic stimulation alters processing of dynamic properties of the peripheral stimulus

I.3.1. Decreased dynamic range for amplitude-dependent responses

In order to analyse further the functional consequences of a 24h period of whisker stimulation onto the response properties in layer IV of the corresponding barrel we decided to quantify single units firing upon a range of PW-deflections varying in the amplitude of the upward movement (angular amplitudes: 0.06 °, 0.18°, 0.30°, 0.68°, 1.02°, 1.43°, 2.1°, ordered form 1 to 7). Electrical activity evoked at the level of the local population of neurons was analysed by means of LFP variations recorded through the electrode at the same time. Recordings were made in barrel C2 of mice of the 24h STIM group (n=4) or in non-stimulated mice (n=5).

General observations. Figure 10.A. illustrates the dependence of cortical responses in layer IV upon the amplitude of the whisker deflection. Upon the deflection of the PW applied with an amplitude of 2.1°, the initial 50 ms component of the LFP is biphasic; a short latency negative phase is followed by a positive phase. The following component of the evoked LFP is a biphasic positive-negative phase. Across trials, i.e. each repetition of the deflection recorded at a same cortical site, and across animals, this temporal pattern of the evoked LFP was highly reproducible. The magnitude of the evoked potentials was also constant across trials; however it varied widely between animals so that only relative voltage magnitudes could be compared.

Figure 10.A. illustrates that with decremented whisker deflection-amplitude the evoked LFP magnitude declines. Neuronal firing is concomitantly decreased with decremented amplitude. The onset and time-to-peaks of the evoked LFP waveform as well as the mode of single unit spiking distribution in function of poststimulus time are delayed with decremented amplitude. Figure 10.A. also shows that at all deflection-amplitudes, the spike incidence reached its maximum during the period of time at which the LFP negative phase was rising. We have observed that the correlation of the onset of the early negative potential with single unit modal latencies recorded simultaneously at the same site was highly significant upon all cells recorded and at each deflection-amplitude ($p<0.0001$ in both group of mice, regression t-test). Moreover, for all significant neuronal responses, i.e. above spontaneous activity, recorded in both group of mice and upon all whisker deflection-amplitudes, modal latencies of single units were invariably comprised between the onset and the peak of the negative potential phase. During the subsequent positive peak of the evoked LFP, single unit firing were usually absent.

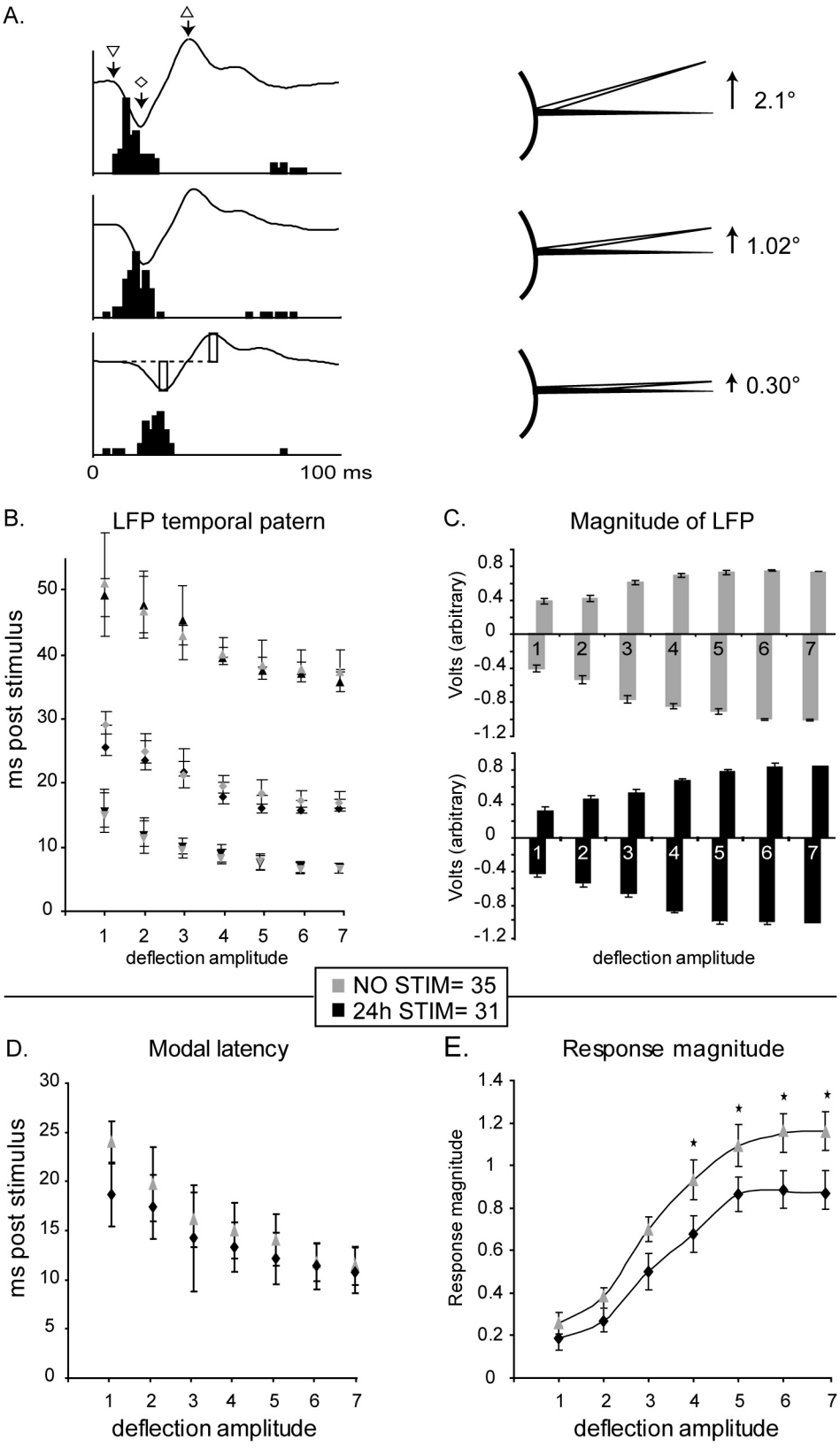


Figure 10. Neuronal response in layer IV of the C2-barrel in function of whisker deflection-intensity in non-stimulated and stimulated mice.

(A) Responses recorded at a single location in layer IV of a non-stimulated mouse for 3 different amplitudes of PW-deflection. Evoked LFP traces and corresponding PSTHs were averaged upon 50 whisker deflections. Evoked response decreased in magnitude with smaller whisker deflection for both single unit firing and LFP. Arrowheads above the upper LFP trace indicate successively the time points for the LFP onset, the peak of the negative phase and of

the positive phase. White bars in the lower diagram indicate the magnitude of the early negative and positive peaks of the LFP. **(B)** Mean ± SD time points of evoked LFP traces. Time to onset, negative and positive peaks of the evoked LFP were correlated to the deflection-intensity. At each intensity, mean time points values were similar in non-stimulated and chronically stimulated mice. **(C)** Mean LFP relative amplitude ± SEM of negative and positive phases upon increasing PW-deflection-intensity. At each location, the average magnitude of the LFP measured towards a given intensity of whisker deflection was expressed relatively to the absolute maximal value of potential recorded at this location within the range of intensity tested. LFP relative magnitudes of the negative and positive phases were correlated with the amplitude of the deflection ($p<0.0001$; regression T-tests). **(D)** Mean single units modal latencies ± SD upon incremental deflection intensities. Layer IV units responded faster with highest deflections ($p<0.0001$). Chronic stimulation did not altered modal latency at any deflection-intensity. **(E)** Mean response magnitude ± SEM of layer IV neurons increased with deflection-intensity ($p<0.0001$). Response magnitudes of layer IV neurons were significantly decreased at the four highest intensity in the 24h STIM group as compared to the NO STIM group. Amplitude of upward movement at deflection intensities 1 to 7: 0.06°, 0.18°, 0.30°, 0.68°, 1.02°, 1.43°, 2.10°.

Correlations of LFP temporal pattern with deflection-amplitudes. At individual recording locations in layer IV, R^2 values (Determination coefficient for regression best fit line) for the correlation of LFP onset with amplitude increments were always comprised between 0.85-0.98, demonstrating that deflection-amplitudes strongly predicted LFP onset time. Figure 10.B. presents mean value of time to onset of the evoked LFP computed over all recordings in NO STIM and 24h STIM animals at each deflection-amplitude. Incremented deflection-amplitudes decreased mean onset time of the LFP response in both group (Regression coefficient for unitary deflection increment ± SE ($b_{y.x}$): -1.4±0.1 and -1.5±0.1 in NO STIM and 24h STIM groups; deflection effect: $p<0.0001$ in both groups, regression t-test). When whisker deflection-amplitude increased, onset time decreased progressively until a minimal plateau at ~6 ms that was reached at 1.02 °or 1.43° deflection-amplitude. The same deflection-response relationships were observed for both time-to-negative and time-to-positive peaks of the evoked LFP: time-to-peaks were negatively correlated with incremented deflection-amplitudes at $p<0.0001$.

Between groups, no differences in the mean values of the time points characterizing the temporal pattern of the LFP were observed at any individual deflection-amplitude. Chronic stimulation did not altered the relationship of time-to-peaks with deflection-amplitude (group*deflection; ANOVA with co-variable).

Correlation of LFP magnitude with deflection-amplitude. The magnitude of the evoked negative and positive field potentials phases were determined by the deflection-amplitude ($R^2>0.6$ at all recording locations). Mean relative magnitudes in function of deflection-amplitudes are presented in figure 10.C. Relative magnitudes of negative and positive peaks augmented with incremented deflection-amplitudes at $p<0.0001$ (regression t-test). The maximal magnitudes of both peaks were commonly reached at deflection-amplitudes of 1.02° or 1.43°. Incrementing amplitude further did not increase evoked LFP amplitudes. The deflection-response relationship was similar in both groups (ANOVA with co-variable). At the lowest amplitude (0.06°), evoked LFP

waveforms were reliably induced in all cases, for both non-stimulated and stimulated mice: mean relative magnitude of LFP peaks were evoked at ~40% of the maximal values for both negative and positive peaks. Even though the lowest deflection-amplitude still evoked activity in layer IV field potentials, we did not systemically assessed responses to deflection of smallest amplitudes. However the smallest amplitude tested was relatively weak (0.06°) and the dependence of voltage amplitudes with deflection was similar along the entire range in both groups, suggesting that the threshold level in layer IV for the detection of whisker upward movement has not been profoundly modified by the chronic stimulation.

Correlation of single units modal latency with deflection-amplitude. Figure 10.D. illustrates the effects of varying the amplitude of the whisker deflection onto mean modal latency ± SD of layer IV single units in non-stimulated and stimulated animals. In both groups, mean modal latency was correlated to the whisker deflection-amplitude ($p<0.0001$): increasing the amplitude accelerated responses of neurons. Chronic whisker stimulation had no effect on single unit latency and did not alter the modulation effect of deflection-amplitude (ANOVA with co-variable).

Correlation of single units response magnitude with deflection-amplitude. As illustrated in figure 10.E., incremented deflection-amplitudes augmented mean response magnitude of neurons in layer IV of stimulated and non-stimulated animals within the range of amplitude used at $p<0.0001$ (regression t-test). The effect of the amplitude was similar in both groups. Chronic stimulation altered the response magnitude of layer IV neurons ($p<0.05$; ANOVA with co-variable). Note that deflection-amplitude determines response magnitude for all individual neurons in both stimulated and non-stimulated animals with all R^2 values >0.6.

Response magnitude was smaller in stimulated animals than in non-stimulated animals at all deflection-amplitudes however these differences did not reach a significant level for responses to PW-deflections < 0.67° ($p<0.05$; MANOVA and Tukey's). The maximal values of response magnitude were reached at deflection-amplitude of 1.43° in both groups of mice. The lowest deflection-amplitude assessed still evoked significant responses above spontaneous activity in two third of the neurons recorded in both groups (24/35 in NO STIM, 25/31 in 24h STIM animals). In both groups, only 2 neurons did not respond after the first increment in deflection-amplitude. Finally, all cells were significantly responsive at higher amplitudes of whisker deflection. These observations indicate that while chronic stimulation modulate response magnitude, it does not altered the sensitivity of layer IV neurons to respond to whisker deflections of small amplitude.

I.3.2. Effects of chronic stimulation onto the adaptation to incremented deflection frequency

We then investigated whether whisker stimulation modifies the dependence of neuronal responses in the corresponding barrel column on the frequency of PW-

deflections. When trains of whisker deflections were applied at various frequencies, we noticed that as soon as interstimulus intervals fall down beneath 500 ms, the number of spikes elicited in a barrel column neuron tends to decrease. At frequencies > 2 Hz, neurons responded to the first deflection in a train at similar level as at low frequencies (0.5 Hz) but less to subsequent deflections, reflecting a steady-state adaptation of responses previously observed in studies made in the barrel cortex of rats (Simons, 1978; Ahissar et al., 2000; Ahissar et al., 2001; Chung et al., 2002). As a result, response magnitude average upon trains of 10 whisker deflections decreased with incremented frequencies. Evoked single unit firing of layer IV and layers II&III neurons in the C2-barrel column of non-stimulated (n=5) and stimulated animals (n=4) were quantified upon 5 trains of 10 PW-deflections for three different frequencies: 0.5 Hz, 4 Hz and 8 Hz.

Correlations of response magnitude with the frequency of PW-deflection. In figure 11, mean response magnitude of neurons in layer IV and layers II&III in the two groups of mice are plotted as a function of deflection frequency. Incremented frequency decreased mean response magnitude of layer IV and layers II&III neurons in both NO STIM and 24h STIM groups of mice between 0.5 Hz and 8 Hz ($p<0.0001$, regression t-test). Response magnitudes of all single units in both groups of mice were strongly determined by the frequency of the deflection with R^2 values always comprising between 0.7-1 (Mean R^2±SD: LIII&II= 0.92±0.10 and 0.90±0.15, LIV= 0.86±0.15 and 0.82±0.14, in NO STIM and 24h STIM mice). Trains of whisker deflection were also applied at higher frequencies (16 Hz): no further decrease in unit firing was observed (for this last comparison, the response of neurons was quantified during a 50 ms period after the stimulus).

Layer IV. Response magnitude of neurons was lower in the stimulated animals as compared to the non-stimulated animals for each frequency of deflections tested. The effects of the 24h period of stimulation were first analysed through a multiple comparison analysis considering responses of layer IV neurons to trains of PW-deflections at 0.5, 4 and 8 Hz as dependent variables. The output of this analysis shows that chronic stimulation significantly reduced response magnitude ($p<0.05$; MANOVA); however the difference between mean response magnitude of responses towards 8 Hz deflections did not reach a significant level (Tukey's). This was presumably due to the small size of the samples (n= 19 in NO STIM and 20 in 24h STIM groups).

In both groups, we observed a similar 2 fold reduction of response magnitude for responses recorded at 8 Hz as compared to 0.5 Hz. The GLM procedure of ANOVA with co-variance did not revealed a significant difference between the effect of the frequency onto response magnitude of layer IV cells in the two groups of animals ($p=0.5305$).

Layers II&III. The response magnitude of layers II&III neurons was significantly altered by the 24h chronic stimulation period at $p<0.05$ (MANOVA). However, while

mean response magnitude of neurons in stimulated animals was strongly depressed towards deflections applied at 0.5 and 4 Hz, the responses to 8 Hz deflections were similar in both groups (Tukey's; p=0.2709). At this highest frequency however, mean response magnitude per deflection was extremely low in both groups. Comparing response magnitude towards 0.5 and 8 Hz, we observed a 4.4 fold reduction in non-stimulated animals and 3 fold reduction in stimulated animals. These observations suggest that the modulation of response magnitude by the frequency of the whisker deflection was different in non-stimulated and stimulated animals. In agreement with that, the GLM procedure of ANOVA with co-variance revealed that the adaptation of neurons with deflection frequency was altered by chronic stimulation (p=.0.0025). This was further supported by the observation that mean slope of the regression lines characterizing the correlation between frequency and response magnitude was significantly decreased in stimulated mice as compared to mice in the non-stimulated group (Mean $b_{y.x}$ ± SD: 0.27±0.1 vs. 0.12±0.7. p=0.0004, two-tailed t-test. Unitary values of increment at each step in the frequency range were used).

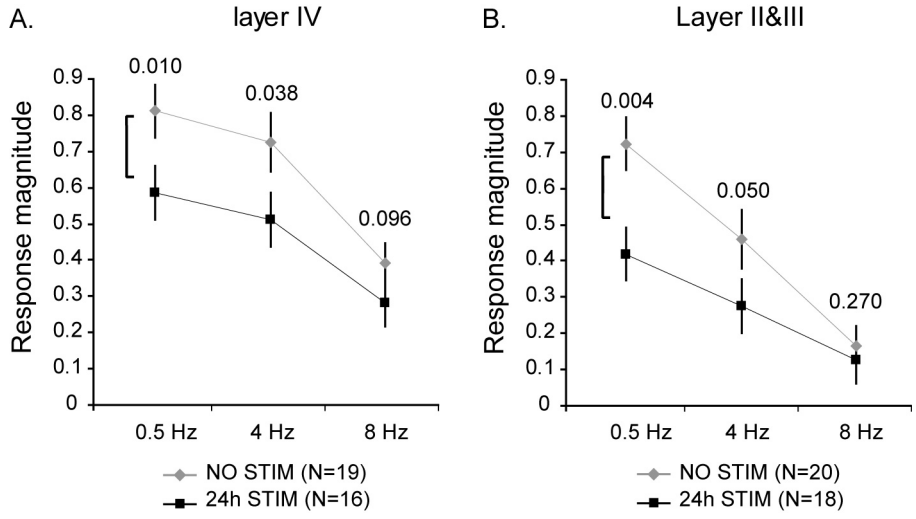


Figure 11. Adaptation of response magnitude to the frequency of PW-deflection in barrel column C2 of stimulated and non-stimulated mice.

(A-B) Mean response magnitude ± SEM for layer IV and layers II&III neurons towards incremented PW-deflection frequencies (0.5, 4, 8 Hz) in non-stimulated (NO STIM) versus stimulated (24h STIM) mice. In both layers, the correlation of response magnitude with the frequency of the deflection was significant (p<0.001). P values calculated for the differences in mean response magnitude between groups at each frequency of deflection are indicated above each graph (MANOVA).

In summary, these analyses revealed a strong adaptation of the responses of neurons in layer IV and in layers II&III of the mouse barrel cortex to the frequency of the PW-deflection, under urethane anaesthesia. In layer IV, chronic stimulation does not alter

the adaptation of neuronal response to the frequency of whisker deflection. However, response magnitude is depressed in the stimulated animals, which lead to a reduction of the dynamic range of frequency-dependent responses. In layers II&III, the dynamic range for frequency-dependent responses is also reduced following chronic stimulation. Moreover, the adaptation of neuronal responses to deflection frequency is lowered following chronic stimulation, which may reflect the fact that stimulus-evoked responses, even to the first stimulus, are already small in layers II&III of stimulated animals.

Part II. Increased expression of astrocytic glutamate transporters in the somatosensory cortex of adult mice after chronic whisker stimulation

II.1. Upregulation of GLT-1 and GLAST protein expression in the stimulated barrel column

Activity-dependent modulation of the relative levels of expression of GLT-1 and GLAST proteins in the barrel cortex was studied by the analysis of 4 independent western blots (see material and methods). Antibodies for GLT-1 and GLAST detected distinct proteins with respectively ~72 and ~ 65 kDa molecular weights (figure 12.A.). To ensure that the levels of expression between the blots were comparable, chemoluminescent bands intensities for both transporters were normalized to the actin (42 kDa) values in each loaded sample. For each gel, the relative levels of GLT-1 and GLAST protein expression in cortical columns C2 from mice of the 24h STIM and 4d-after-STIM groups were then expressed as percentages of the levels measured in mice of the NO STIM group (=100%). Results of this analysis are shown in figure 12.B., presenting mean ± SD relative transporters expression values in percent of NO STIM, calculated from the four independent experiments. After a 24h period of chronic C2-whisker stimulation, GLT-1 and GLAST relative expressions in the C2-barrel column were increased by 265±38% and 246±20%, respectively, as compared to NO STIM values ($p<0.05$, Tukey's). Four days after the cessation of the stimulation, the relative levels of both glutamate transporters were not significantly different from that in non-stimulated mice (GLT-1: 103±12%; $p=0.89$; GLAST: 75±7%, $p=0.27$).

II.2. Unchanged expression of glutamine synthetase (GS) after chronic whisker stimulation

A second set of experiments was realized to measure whether the activity-dependant increase in astrocytic glutamate transporters was accompanied by a modulation in the level of expression of GS, the glial enzyme predominantly responsible for the glutamate recycling after its uptake by converting it to glutamine. Three independent immunoblotting experiments were realized. Each time, GS immunofluorescence intensities were measured together with GLT-1, in order to ensure that glutamate transporters were actually increased by whisker stimulation in these experiments, and actine. The antibody for GS detected a distinct 45 kDa molecular weight protein

(figure 13.A.). The level of GS expression was not significantly modified by the chronic stimulation (112±38% and 99±4% in 24h STIM and 4d-after-STIM as compared to NO STIM). Immunoblots quantification (figure 13.B.) confirmed that GLT-1 was increased by 251±39% after a 24h period of stimulation as compared to values in non-stimulated animals. Mean GLT-1 expression in barrel samples from mice of the 4d-after-STIM group was not significantly different than in the NON STIM group (98±5%). Both measurements are comparable to results obtained in the experiments described in the previous paragraph.

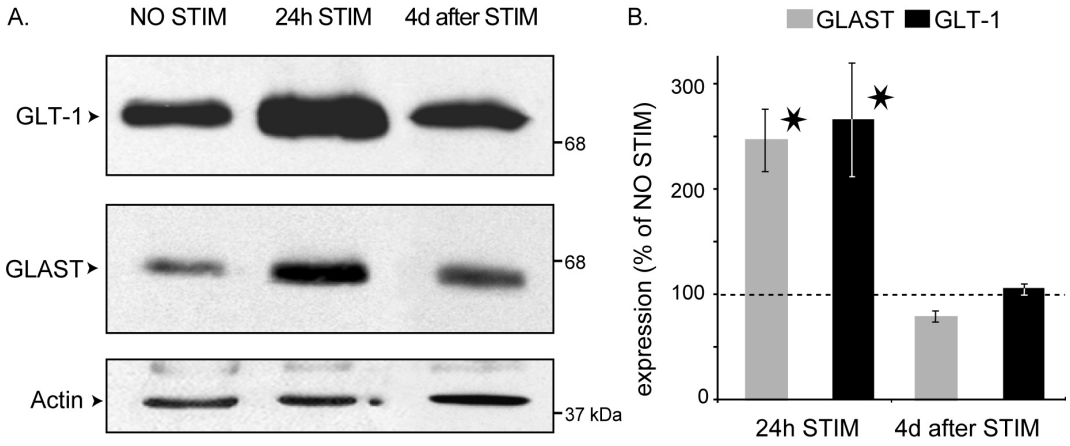


Figure 12. Upregulation of GLT-1 and GLAST protein levels following a 24h period of whisker stimulation.

(A) Representative immunoblots for glutamate transporters GLT-1 (72 KDa) and GLAST (65 KDa) and for actin (42 KDa) obtained from barrel columns C2, dissected immediately after a 24h period of C2-whisker stimulation (24h STIM), 4 days after (4d-after-STIM) or in non-stimulated mice (NO STIM). Blot was probed for GLAST, GLT-1 and actin and revealed a clear increase in GLAST and GLT-1 levels. For each experimental condition, two barrel columns were used. Each dissected barrel column had been identified to be the C2-barrel column in the Nissl-stained tangential sections. Migration levels of the markers for molecular weights are indicated in KDa. **(B)** Results of GLAST and GLT-1 levels on immunoblots from four independent experiments were quantified by densitometry. Values were normalized to the level of actin. In each gel, optical intensities measured for 24h STIM and 4d-after-STIM samples were then expressed as percentages of levels measured for NO STIM samples (=100%), used as control. GLAST and GLT-1 mean levels in the C2-barrel column were significantly increased by 246±20% and 265±38%, respectively, following 24h of whisker stimulation as compared to levels in non-stimulated mice. Levels of expression of both proteins in samples extracted 4 days after the stimulation was halted were not significantly different from samples of non-stimulated mice. These results demonstrated a strong but transient modulation of glutamate transporters by neuronal activity in vivo. Asterisks indicate $p<0.01$ (Tukey's), error bars are SD.

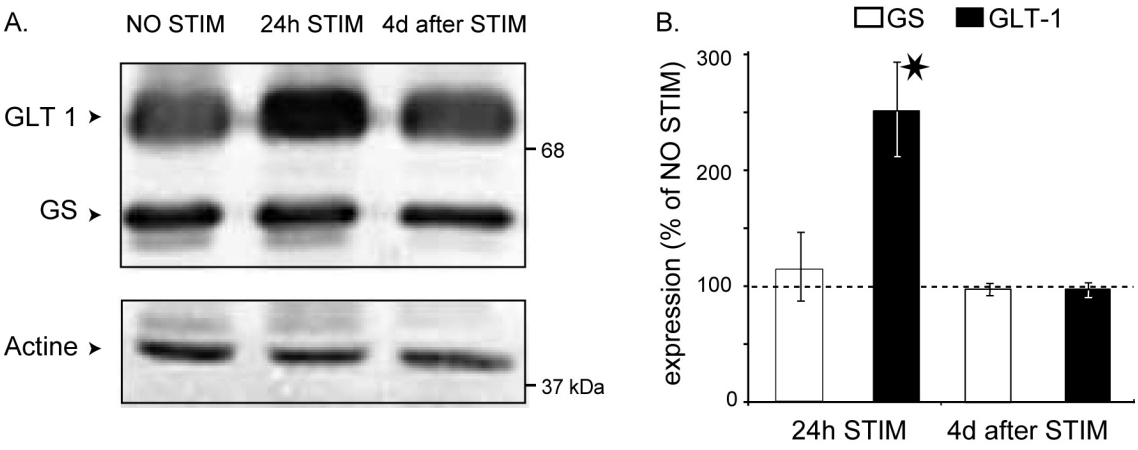


Figure 13. Expression of glutamine synthetase protein (GS) is not modulated by chronic stimulation, as measured by immunoblots.

(A) Representative immunoblots for glutamate transporter GLT-1, GS (45 KDa) and actin (42 KDa) from three pools of two barrel column C2, dissected in mice immediately after a 24h period of C2 whisker stimulation (24h STIM), 4 days after (4d-after-STIM) or in non-stimulated mice (NO STIM). Migration levels of the markers for molecular weights are indicated in KDa. **(B)** Densitometry results expressed in mean ± SD from three independent experiments. In each gel, optical intensity values for GLT-1 and GS were normalized to actin levels then ratios were calculated as 24h STIM or 4d-after-STIM / NO STIM (= control:100%) values. The expression of GLT-1 after a 24h period of C2 stimulation was significantly increased by 251±39% as compared to control and returned to control values 4 days after, as in the tissues analyzed for figure 12. The level of GS remained unchanged after the prolonged whisker stimulation period. Asterisk indicates $p<0.01$ (T-test), error bars are SD.

II.3. Increased expression of GLT-1 and GLAST proteins in the stimulated barrel column as compared to a non-stimulated one

In the experiments described so far, the modulation of the level of transporters protein expression after a prolonged period of whisker stimulation was studied by comparing relative levels between barrel column C2 in stimulated mice and that of the same barrel in non-stimulated mice. In a supplementary experiment, we have extracted both the C2-barrel column and the E2-barrel column in the PMBSF of the right hemisphere of one mouse that had been prior submitted to a period of 24h stimulation of its contralateral C2-whisker. Relative levels of GLT-1 were then compared between the stimulated C2-barrel column and the non stimulated E2-barrel column. Figure 14.A. shows a photomicrograph of a 40 μm tangential section of the PMBSF confirming that the C2- and the E2-barrel columns, corresponding to the stimulated C2-whisker and the non-stimulated E2-whisker, had been collected. Semi-quantification of the immunoblot shown in figure 14.B. revealed a higher level of relative GLT-1 band intensity in the extract from the stimulated barrel column as compared to the extract form the E2-barrel column collected in the same mouse (220% increase), as well as compared to the expression in the C2-barrel extract from a non-stimulated animal (170% increase).

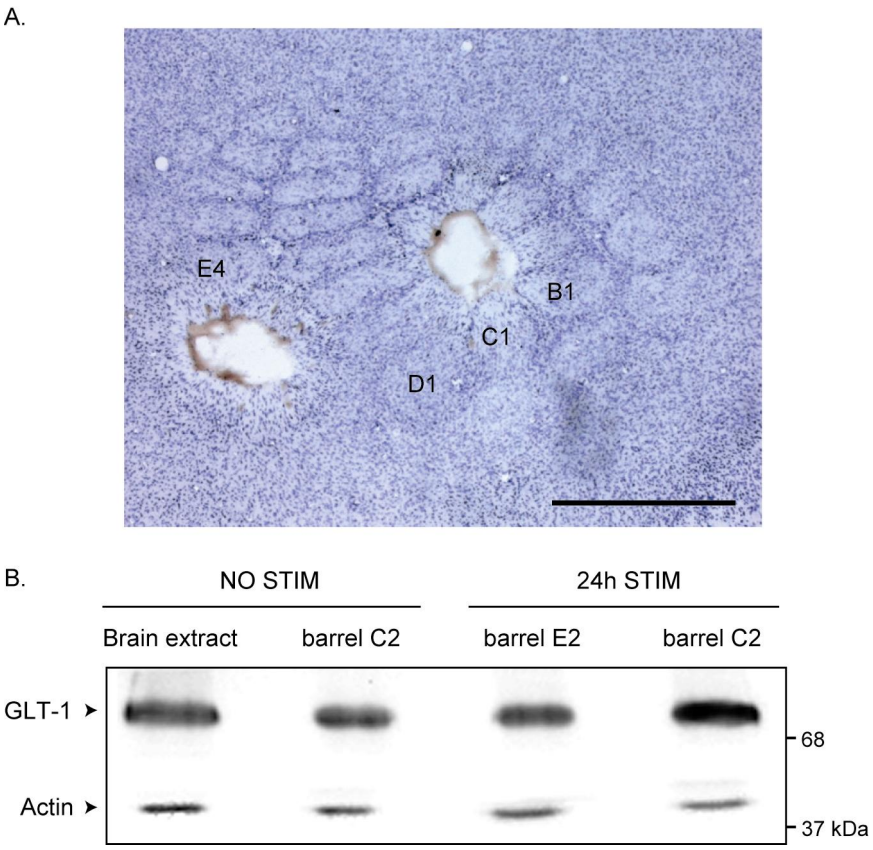


Figure 14. GLT-1 protein expression in a "stimulated" barrel column versus a "non stimulated" barrel column dissected from the same hemisphere.

(A) Photomicrograph of a Nissl-stained tangential layer IV section from a mouse in which both the C2- and the E2-barrel column were dissected, immediately after a 24h period of C2-whisker chronic stimulation. (B) These two samples were homogenized, denaturized and loaded on the same electrophoresis gel (24h STIM), together with an extract of whole cortex and of a C2-barrel column dissected in a non-stimulated mouse (NO STIM). The resulting immunoblot for GLT-1 and actin is presented here; relative to actin values, the optical density measured for GLT-1 bands was observed higher in the sample extract from the C2 barrel column of the stimulated mouse as compared to the sample from the E2 barrel column of the same mouse as well as to the two samples from non-stimulated mice. GLT-1 relative expression was increased by the stimulation whatever the control used: 220% increase as compared to the E2-barrel column collected in the same mouse, 170% increase as compared to expression in the C2-barrel of a non-stimulated animal. (Scale bar is 500 µm)

DISCUSSION

Using *in vivo* electrophysiological extracellular recordings, the present study shows that sensory stimulation evokes neuronal plasticity in the somatosensory cortex of the adult mouse. In a previous study made in our laboratory, it was shown that a 24h period of chronic whisker stimulation induces an increase of the number of GABAergic synapses in the stimulated barrel (Knott et al., 2002). At a functional level, we report here that chronic whisker stimulation induces changes in the response properties of cortical neurons. We propose that these experience-dependent changes of neuronal responses: 1) correlate with the structural synaptic changes; and 2), point to a mechanism of habituation to the chronically stimulated whisker. Furthermore, by means of a semi-quantitative immunoblotting technique, we show that chronic whisker stimulation induces experience-dependent plasticity in glial cells, with an upregulation of the expression of astrocytic glutamate transporters specific to the stimulated barrel column.

A. Modified responses to the chronically stimulated whisker

I. Unaltered spatiotemporal pattern of evoked activity in the stimulated barrel column

The deflection of the PW generates neuronal activity in the corresponding barrel column that starts in layer IV, followed by a sequential processing across the cortical layers. This sequential laminar processing of sensory signals can be revealed by measuring the LFP evoked by the PW at various depths in a barrel (Mitzdorf, 1985). The LFPs recorded at discrete location are generated by current generators called sinks and sources. These current sinks and sources originate mainly from local changes in neuronal membrane conductance created by synaptic activation and less by those created by propagated APs (Mitzdorf, 1985; Leung, 1990). The CSD derivatives of the LFPs evoked at various depths in a barrel column allows the determination of the time course and location of current sinks and sources (Nicholson and Freeman, 1975; Mitzdorf, 1985). The deflection of the PW evokes a highly reproducible current sink of ~20 ms duration at the level of the layer IV and upper layer V with an onset of 5-7 ms poststimulus. This early sink invades layers II&III within 1-3 ms later and is concomitant with current sources in the superficial and deep layers. During the next 25 ms, the early sink is followed by a current source with onset around 25 ms and lasting

until 50 ms poststimulus concomitant with a current sink in deep layers, peaking in the middle of layer VI.

Previous CSD experiments made in rats have shown that the initial negative going phase of the cortical LFPs evoked in layers IV - II&III by the PW reflects the current sink in those layers (Di et al., 1990; Castro-Alamancos and Oldford, 2002). Current sinks are thought to be governed mainly by excitatory postsynaptic currents (EPSCs) (Leung, 1990; Mitzdorf, 1985). It was shown in cortical slice preparations that the negative going phases of evoked LFPs are dependent upon AMPA and NMDA glutamate receptors (Wirth and Luscher, 2004). Thus, the early current sink that peaks in layer IV reflects the excitatory drive of the thalamocortical projections (White, 1978; Agmon and Connors, 1991; Agmon and Odowd, 1992) from an individual barreloid that arborize mainly in the corresponding barrel (Killackey, 1973; Frost and Caviness, 1980; Jensen and Killackey, 1987; Bernardo and Woolsey, 1987; Chmielowska et al., 1989).

The flow of excitation that spreads in layers II&III and subsequently invades infragranular layers is in agreement with the known excitatory output of layer IV to supragranular layers (Feldmeyer et al., 2000; Lubke et al., 2000; Petersen and Sakmann, 2001) and the descending projections from both layer IV and layers II&III neurons to infragranular layers (Harris and Woolsey, 1983; Gottlieb and Keller, 1997; Zhang and Deschenes, 1998). Thus, the present CSD analysis is coherent with the laminar sequential processing model and the generally postulated "VB→layer IV→layers II&III→layers V/VI" pathway (Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Welker et al., 1993).

The current source that follows the early sink in layer IV may be driven by feedforward and feedback inhibition from layer IV inhibitory interneurons (Swadlow and Gusev, 2000; Porter et al., 2001). This current source temporally matches a positive LFP phase that peaks in layer IV. Wirth and Lusher (2004) have shown that the positive LFP phase that interrupt the negative LFP phase evoked by the activation of layer IV is dependent on the activation of $GABA_A$ receptors.

Neither the laminar location, the onset latency nor the duration of the early current sink evoked by whisker deflection is altered after chronic whisker stimulation. The subsequent pattern of sink-source distributions is also conserved. Thus, the present CSD analysis reveals that the activation of a cortical column initiated by its TC-inputs is not altered by chronic whisker stimulation in its spatial and temporal characteristics. However, quantitative modifications in the amplitude of the signals measured in the cortex cannot be excluded neither on a population nor on a single cell level.

II. Depression of single unit responses in the stimulated barrel column

II.1. Characteristics of PW-evoked single unit responses in layer IV

Latencies. In the barrel cortex of rodents layer IV acts as a cortical gate for the stream of peripheral sensory signals. The first cortical neurons to fire action potentials (APs) upon striking a whisker are located in layer IV of the corresponding barrel column (Simons, 1978; Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Welker et al., 1993). In agreement with that, layer IV neurons have the shortest latency (5-9 ms) for PW-evoked excitatory post synaptic potentials (EPSPs) in rats, as compared to neurons in other cortical layers (Carvell and Simons, 1988; Moore and Nelson, 1998; Zhu and Connors, 1999; Wilent and Contreras, 2004).

Sensory signals evoked by the PW stimulus cross at least two synapses in the brainstem and the thalamus to reach layer IV neurons through the lemniscal pathway. Under similar conditions of anaesthesia as in the present study, it has been shown In rats that upon striking the PW, barrel hollow cells generate APs with a mean modal latency of 8-9 ms (Armstrong-James and Fox, 1987; Armstrong-James et al., 1992; Petersen and Diamond, 2000). In mice, our study shows mean modal latencies towards the PW of ~10-11 ms in layer IV (See figure 5). The higher mean value of modal latency in mice as compared to rats (~2 ms) presumably reflects differences in conduction velocity or synaptic delays in the lemniscal pathways of the two species. About 50% of layer IV neurons have short modal latencies (<12ms) in both non-stimulated and stimulated mice, indicating that they should respond monosynaptically to TC activation. Chronic whisker stimulation does not modify the latency of neuronal responses, in agreement with our CSD analysis showing that the temporal characteristics of cortical activation are not altered.

PSTH-profile. The deflection of the PW induces a robust increase in AP spiking probability among the layer IV neuronal population that reaches a maximum 11 ms poststimulus and then gradually decreases to near spontaneous levels ~25 ms poststimulus. A period during which evoked firing is almost absent follows, lasting until 50 ms poststimulus. This profile of evoked spiking precisely matches the timing of the current sink-source sequence in layer IV. A second period of AP spiking subsequently takes place between 50-100 ms after the stimulus. A similar profile of sensory evoked responses of layer IV neurons consisting in a bi-phasic spike activity pattern was already reported in the somatosensory cortex of the anaesthetized rats (Simons and Land, 1987; Armstrong-James and George, 1988; Simons and Carvell, 1989; Simons et al., 1992). Other studies have reported in rats and mice that after an initial period of high evoked activity, neuronal activity decrease towards a low level of spiking above spontaneous rate that is consistently sustained throughout the entire duration of the response to the PW (Welker et al., 1993; Armstrong-James et al., 1993). These discrepancies in PW-response profiles may reflect differences in the level

of anaesthesia, with biphasic profile being more prevalent during light anaesthesia (Armstrong-James and George, 1988). Chronic stimulation does not affect this profile of evoked spiking.

Thalamocortical processing of sensory signals. Upon PW-deflection, the earliest spikes recorded in layer IV are monosynaptic responses to the TC-volley that depend on the activation of AMPA-Rs; however NMDA-Rs are also engaged by TC-activation (Armstrong-James et al., 1993; Gil and Amitai, 1996; Fleidervish et al., 1998). Following layer IV activation by TC-inputs, evoked neuronal spiking is then rapidly mediated by excitatory interactions of cortical origin that depend almost entirely on NMDA-Rs activation (Armstrong-James et al., 1993; Fleidervish et al., 1998). In mice, a particular subtype of NMDA-Rs specific of layer IV neurons exhibit low voltage-dependence and can support APs without previous depolarization (Fleidervish et al., 1998).

The response characteristics of layer IV cortical neurons are determined by the dynamic, the pattern and the relative strength of their inputs from TC and intracortical (IC) excitatory synapses and by the local inhibitory network. In mice, each barrel contains an heterogeneous population of ~2000 neurons (Lee and Woolsey, 1975). Of these, ~80% are glutamatergic, mainly spiny stellate neurons and some "modified pyramidal cells" (White, 1978; White and Rock, 1980a; Harris and Woolsey, 1981; Harris and Woolsey, 1983). The dendritic field of spiny stellate cells in both mice and rats are confined to the barrel in which their somata are located; their axons strongly ramify within layer IV and project to other cortical layers in a highly columnar fashion (Harris and Woolsey, 1983; Feldmeyer et al., 1999; Lübke et al., 2000; Petersen and Sakmann, 2000). The other ~20% of the barrel neurons are GABAergic inhibitory aspiny neurons of various morphological types (Porter et al., 2001). Their dendritic and axonal fields are also largely restricted to layer IV but axonal arbors often extend to supragranular layers and sometimes to adjacent barrels (White, 1978; Simons and Woolsey, 1984; Porter et al., 2001). Both populations receive TC-synapses (White, 1978; Benshalom and White, 1986; Keller and White, 1987) and respond monosynaptically to the TC-volley (Gil et al., 1999; Porter et al., 2001)[2]. All TC-synapses are asymmetrical and glutamatergic (Keller et al., 1985; Agmon and Odowd, 1992). A layer IV neuron receives between 5-20 % of its excitatory synapses from TC-axons, the others originate mainly from spiny stellate neurons in the same barrel (White, 1978; White and Rock, 1980b; Benshalom and White, 1986) and to a lesser

[2] *Evoked AP firing that we have recorded from layer IV single units most likely represents the responses of both excitatory and inhibitory cells. Two populations of extracellular spikes differentiated by their waveforms can be recorded in the cortex: short duration "thin" spikes and long duration "regular" spikes. It is thought that thin spikes arise from smooth stellate GABAergic neurons and that regular spikes originate from excitatory neurons (Mccormick et al., 1985;Connors and Gutnick, 1990;Welker et al., 1993). In the present study, two third of layer IV neurons were characterized by a thin spike in all groups. However, "thin" and "regular" spike neurons were pooled together to avoid misinterpretations of our results because of uncertainties about classifications of neurons.*

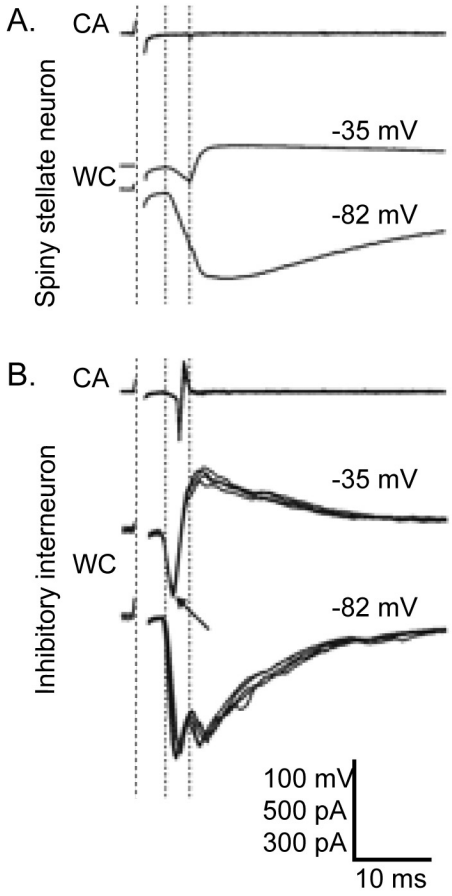
extent from neurons in layer VI of the same column (White, 1989; Zhang and Deschenes, 1997; Schubert et al., 2003).

Gil and colleagues (1999) have estimated the synaptic efficacy of TC and cortico-cortical inputs onto layer IV neurons using an *in vitro* thalamocortical slice preparation from the mice brain. They showed that the connection established by a single TC-axon with a layer IV spiny stellate neuron is ~4.8 fold more effective than any connection from another spiny stellate neuron within the same barrel, due to a greater number of release sites and a strongest release probability. The high efficacy of TC-synapses should ensure a robust impact of TC-activation in layer IV neurons. The number of synapses established between neurons of a connected pair of spiny stellate layer IV neurons was estimated to 1-4 (Gil et al., 1999). A similar connectivity was demonstrated in reconstructed pairs of spiny stellate neurons of young rats barrel cortex with 2-5 synapses per connected pairs (Feldmeyer et al., 1999; Lübke et al., 2000). It has been estimated that each spiny stellate neuron is monosynaptically connected with ~75 neurons within a barrel (Fleidervish et al., 1998). Physiologically, connections between spiny stellate neurons within barrels were shown to be highly reliable (Feldmeyer et al., 1999; Gil et al., 1999; Beierlein et al., 2003). Moreover, bi-directional synaptic connections and autapses were observed in spiny stellate neurons (Feldmeyer et al., 1999). Thus, barrels contain the anatomical and functional substrate for a strong recurrent excitatory network that will be activated by the TC-volley. The densely interconnected excitatory network presumably mediate a selective amplification of afferent thalamic signals before its propagation via layer IV axon collaterals throughout the cortical column (Harris and Woolsey, 1983; Douglas et al., 1995; Feldmeyer et al., 1999; Petersen and Sakmann, 2000; Cowan and Stricker, 2004).

Upon the striking of a whisker (Simons and Carvell, 1989; Welker et al., 1993; Bruno and Simons, 2002) or following electrical stimulation of TC-afferents (Porter et al., 2001; Beierlein et al., 2003), inhibitory neurons in layer IV are more susceptible to fire APs, have shorter latencies and higher evoked spiking rates in comparison with excitatory neurons. It has been reported from intracellular studies in mouse TC-slice preparations that the efficacy of TC-synapses on inhibitory neurons is 5 fold stronger than on excitatory (Porter et al., 2001). The stronger activity evoked in inhibitory neurons could rely on the observation that TC-synapses contact somata and proximal dendrites of inhibitory neurons while in excitatory neurons TC-synapses are made on dendritic spines (Staiger et al., 1996a; Keller and White, 1987). Stronger TC-synaptic currents might also be engaged in inhibitory neurons due to a particular AMPA-subtype receptor channel of high conductance in their postsynaptic membrane (Angulo et al., 1997). As suggested by observations made in the young (P14-P21) rats barrel cortex (Gibson et al., 1999; Szabadics et al., 2001), electrical coupling through gap junctions should further enhance the synchronicity of the activation of inhibitory neurons.

These observations indicate that inhibitory interneurons should generate strong feedforward inhibition upon TC-activation. In agreement with that, it has been shown that upon the deflection of the PW in anesthetized rats (Carvell and Simons, 1988; Moore and Nelson, 1998; Zhu and Connors, 1999; Higley and Contreras, 2003; Wilent and Contreras, 2004) or the stimulation of VB neurons in TC-slice preparations of the mouse brain (Agmon and Connors, 1992; Gil and Amitai, 1996; Porter et al., 2001), an EPSP is evoked at the soma of virtually all layer IV neurons of the corresponding barrel and followed within 5 ms by an early inhibitory postsynaptic potential (IPSP). This early IPSP, presumably mediated by $GABA_A$-Cl^- receptors (Kyriazi et al., 1996a; Gil et al., 1997), curtails the EPSP as soon as 10-11 ms after the EPSP onset. It is followed by a long-lasting K^+-dependent IPSP, mediated by G-protein-coupled $GABA_B$ receptors (Kyriazi et al., 1996a). Evoked inhibition is often followed by a second EPSP at variable latencies (50-200 ms poststimulus). Deflection of SWs also generate IPSPs ("surround inhibition") however the strongest inhibition is always evoked by the PW (Moore and Nelson, 1998). Surround inhibition, whether mediated by intra-barrel or trans-columnar inhibitory connections, has been proposed as the mechanism by which SWs could mediate cross-whisker suppression (Simons, 1985; Simons and Carvell, 1989; Moore and Nelson, 1998; Porter et al., 2001; Higley and Contreras, 2003). Figure 15 shows an example of the subthreshold response evoked by the activation of TC-afferents in a spiny stellate and an inhibitory neuron (Porter et al., 2001). The inhibitory interneuron exhibits a monosynaptic excitatory postsynaptic current (EPSC) that peaks slightly before the EPSC recorded in the spiny stellate neuron and fires an AP 1.2 ms before the onset of the inhibitory postsynaptic current (IPSC) in the spiny stellate neuron. Thus, the timing of the AP evoked in the inhibitory interneuron suggests it mediates feedforward inhibition in spiny stellate neurons. Porter and colleagues (2001) have demonstrated that the different GABAergic subpopulations of layer IV neurons they observed could all mediate feedforward inhibition upon TC-activation.

Strong feedforward inhibition should rapidly tend to hold layer IV neurons membrane potential below spiking threshold, defining a short temporal window for the processing of excitatory response. The nature and the temporal pattern of the subthreshold events generated in individual neurons are consistent with the profile of spiking evoked by the PW that we have observed in layer IV populations of neurons, i.e. an initial rapid peak in AP firing followed by a period during which evoked AP firing is almost absent and then a second period of low evoked firing.



Adapted from Porter et al (2001)

Figure 15. Examples of subthreshold responses in layer IV neurons: inhibitory neurons mediated disynaptic inhibition
Subthreshold responses in two layer IV neurons towards the stimulation of the VB nuclei of the thalamus in a mouse thalamocortical slices preparation (Porter et al, 2001). **(A)** Spiny stellate neuron. Top traces: four superimposed cell-attached responses (CA) with no detectable spikes recorded form the same cell. Bottom traces: average whole-cell responses (WC) towards stimulation of thalamocortical neurons while displacing the Vm at -35 mV and -82 mV to examine the synaptic composition of the neuronal responses. The time of the thalamocortical stimulation is indicated by the first guideline. The spiny neuron receives a monosynaptic excitatory postsynaptic current (EPSC; onset indicated by the second guideline) following by a disynaptic inhibitory postsynaptic potential (IPSC) that reversed between -82 mV and -35 mV (onset of the IPSC indicated by the third guideline). **(B)** Inhibitory interneuron. Guidelines as in A. Top traces: four superimposed CA responses showing that this neuron fired spikes consistently. Bottom traces: five superimposed WC responses. An early EPSC is followed by a dual IPSC. The onset of the early IPSC is indicated by the arrow, the second IPSC has the same onset time than the IPSC of the spiny stellate neuron in A. The spiking of the inhibitory neuron at monosynaptic latencies after the thalamocortical volley and the onset time of IPSCs in both cortical neurons, which were recorded in the same barrel, indicate that inhibitory neurons are well suited to evoke disynaptic inhibition in surrounding neurons.

The inhibitory networks serves several functions during cortical processing, from the shaping of the spatiotemporal features of cortical representations to the control of excessive excitation within the cortical network (Simons, 1978; Dykes et al., 1984; Kyriazi et al., 1996b; Kyriazi et al., 1998; Miller et al., 2001; Zhu et al., 2004). Importantly, strong feedforward inhibition is likely to enhance the contrast between "non-preferred" and "preferred" inputs: following a dampening model, fast inhibition could render the barrel excitatory circuitry particularly sensitive to TC-inputs that are initially highly synchronized, i.e. preferred inputs, and insensitive to less synchronized, potentially distracting, inputs (Kyriazi and Simons, 1993; Welker et al., 1993; Pinto et al., 2000; Beierlein et al., 2002; Wilent and Contreras, 2004). In another model, inhibition was proposed to keep recurrent excitation proportional to the stimulus strength (Douglas et al., 1995).

II.2. Depression of PW-evoked responses in layer IV

Transient and localized down-regulation of spontaneous activity and PW-response magnitude. Quantitative measurements of single unit spiking reveal that after a 24h period of chronic whisker stimulation, spontaneous activity decreases by 44% in the stimulated barrel. Mean response magnitude (RM) of neurons towards the deflection of the stimulated whisker decreases by 25% in the stimulated barrel (See figure 16). RM, which represents the number of spikes evoked per whisker deflection during the 100 ms post-deflection, was corrected for spontaneous activity. Mean RM of neurons in non-stimulated barrel columns C1 and D2 towards the deflection of their own PWs is not altered. Similarly, the decrease in spontaneous activity is only observed in the stimulated barrel. Thus, both the decrease in spontaneous activity and the diminished PW-activation are restricted to the stimulated barrel. Those experience-dependent changes are transient as spontaneous activity and RM were no longer depressed in the stimulated barrel 4 days after the cessation of the stimulation. These results demonstrate that a 24h period of modified sensory experience at the level of a single whisker can induce functional changes of neuronal activity in layer IV of the corresponding barrel.

Altered dynamic range for stimulus intensity and frequency. Neuronal responses in the sensory cortices are sensitive to several parameters of the stimulus, allowing the brain to represent, or encode, specific features of the environment (Mountcastle, 1957; Hubel and Wiesel, 1962; Simons, 1978). Due to the intrinsic properties of the piezoelectric device, the velocity of the whisker movement actually vary along with amplitude (Armstrong-James and Fox, 1987), therefore the variations of these parameters will be referred here after as changes in stimulus intensity. As previously reported in rats (Armstrong-James and Fox, 1987; Peterson et al., 1998; Pinto et al., 2000; Wilent and Contreras, 2004), we have observed that single unit modal latency decreases while RM robustly increases with incremented deflection-intensity. This functional relation between stimulus intensity and neuronal response in layer IV was

also observed using LFP analysis and is not affected by the chronic stimulation (GLM Regression analysis). It has been proposed that the stronger responses of layer IV neurons at highest deflection intensities reflect stronger and more synchronized TC-inputs and their transformation by the layer IV network (Pinto et al., 2000; Shoykhet et al., 2000; Wilent and Contreras, 2004). Mean RM of neurons is lowered at all deflection intensities in stimulated mice as compared to non-stimulated mice; although this effect is statistically significant only at the highest intensities. At the lowest intensity tested, PW-deflections evoke low level of responses but still above spontaneous activity in both the non-stimulated and stimulated groups of animals. The lowest deflection-intensity tested could be considered as near-threshold because first, it induces an upward deflection of only 0.06°, close to the threshold values estimated in rats by Peterson et al. (1998). Second, mean RM of layer IV neurons is extremely weak at this deflection-intensity, barely exceeding 0.1 spikes per deflection, with one third of neurons being unresponsive in both groups of mice. RM of layer IV neurons could vary from virtually 0 at a weak level of deflection-intensity to an average of 1.18 spikes per deflection in non-stimulated and 0.85 spikes per deflection in stimulated animals, both values similarly reached at the deflection-intensity level characterized by an angular upward movement of 1.02°. Altogether, these results indicate that chronic stimulation does not profoundly modify the threshold level for the detection of whisker movement but decrease the dynamic range of the cortical response for deflection-intensity.

The effect of chronic stimulation onto the short-term dynamic of neuronal responses was also examined by varying the frequency of the stimulus from 0.5 to 8 Hz. Several previous studies have shown that under anaesthesia, whisker-evoked spiking of layer IV neurons decreases at frequencies of deflections >2 Hz reflecting a steady-state adaptation of responses: the number of spikes evoked by consecutive deflections decreases during the train of deflection (Simons, 1978; Ahissar et al., 2000; Ahissar et al., 2001; Chung et al., 2002). This adaptation of cortical neurons responses results principally from short term depression at TC-synapses and at excitatory synapses between layer IV neurons (Gil et al., 1997; Chance et al., 1998; Egger et al., 1999; Finnerty et al., 1999; Sosnik et al., 2001; Petersen, 2002; Chung et al., 2002) and might play an important role when rodents actively move their whiskers at frequencies ~8 Hz during exploration behaviour. In mice, our study shows that RM decreases with incremented frequency between 0.5 and 8 Hz. For each frequency, RM was averaged upon 5 trains of 10 deflections separated by 2 seconds. In both non-stimulated and stimulated animals, mean RM of layer IV neurons towards deflections delivered at 8 Hz shows a 2 fold reduction as compared to RM towards deflections delivered at 0.5 Hz. The absence of a difference between adaptation in chronically stimulated and non-stimulated animals indicates that the short term dynamics at TC and cortico-cortical inputs within layer IV are presumably unchanged after chronic stimulation. However, given that RM is lower in stimulated animals, the dynamic range of frequency-dependent responses is decreased after chronic stimulation.

Altogether, the transient decrease in spontaneous activity and PW-evoked responses in the stimulated barrel correlates well with the decrease in DG-uptake previously observed following chronic stimulation by Welker et al (1992). The decrease in the dynamic range of deflection-intensity- and frequency-dependent responses may affect the retrieving and encoding of stimulus clues critical for detection of stimuli, discrimination of surface textures and distance to objects (Moore et al., 1999; Petersen, 2002; Moore, 2004). These experience-dependent effects suggest a habituation-like mechanism induced by the prolonged stimulation of a whisker.

II.3. Possible mechanisms of experience-dependent changes in layer IV following chronic stimulation

Cortical versus subcortical origin of plasticity. The diminished response magnitude of layer IV neurons in the stimulated barrel could originate from a decrease in the activity evoked in the subcortical stations of the whisker-to-barrel pathway, whether resulting from follicular receptors "fatigue" or to experience-dependent plasticity at the level of the brainstem and the thalamus or at the TC connections. Alternatively, the depression of PW-responses could also result from changes at the level of cortico-cortical synaptic transmission within the stimulated barrel itself. To determine at which step of the processing the depression of evoked activity first occurs, we have processed to a quantitative epoch analysis of layer IV neuronal responses towards PW-deflection delivered using the standard protocol. This analysis revealed that PW-response is not affected by the chronic stimulation during the earliest post-deflection time epoch (T1: 3-11 ms), whereas it is significantly smaller during the second time epoch (T2: 12-25 ms).

Neuronal firing during T2 should be predominantly driven by cortico-cortical rather than by the thalamocortical excitatory connections. A barreloid neuron can often discharge more than 2 spikes at 300-400 Hz following the deflection of the PW (Simons and Carvell, 1989; Kyriazi et al., 1994; Pinto et al., 2000). Under urethane anaesthesia in rats, the period of strongly synchronised spiking of barreloid neurons peaks 5-6 ms after the stimulus and lasts between 4 and 10 ms poststimulus (Diamond et al., 1992; Armstrong-James and Callahan, 1991; Glazewski et al., 1998b). Thus, the strength of the TC-volley is strongly reduced as soon as 9-10 ms after the stimulus, presumably because of the strong inhibition generated by the reticular nucleus (Brecht and Sakmann, 2002b). Taken this to the mouse, the unaltered T1 response after chronic stimulation indicates that layer IV receive a TC-activation that is not modified compared to non-stimulated animals. However, the diminished spiking during T2 indicates a mechanism of plasticity that has its origin in the transmission within the cortico-cortical synaptic network in the stimulated barrel. Quantification of evoked firing in barreloid neurons of stimulated and non-stimulated mice will be useful to confirm this assumption.

Several studies have shown in adolescent rats that innocuous modifications of sensory experience cause substantial plasticity in the sensory cortex but not in the subcortical stations of the sensory pathway (Glazewski et al., 1998b; Wallace and Fox, 1999; Wallace et al., 2001). It has been established that experience-dependent receptive fields changes in the adult barrel cortex of rodents are mediated by synaptic plasticity occurring predominantly at intracortical synapses (Armstrong-James et al., 1994; Rema et al., 1998; Barth et al., 2000; Wallace et al., 2001; Buonomano and Merzenich, 1998; Foeller and Feldman, 2004 for a review). Nevertheless, TC synaptic plasticity might still be inducible in the adult rat upon long periods of modified sensory experience: while 3-10 days of whisker-pairing induce an increase in the PW-responses in spared barrels that is confined to the late time epochs of the PSTHs, neuronal firing is potentiated during the early time epoch of the responses after 30 days of whisker-pairing (Diamond et al., 1993; Armstrong-James et al., 1994). Moreover, chronic whisker stimulation leads to a decrease in 2-DG-uptake at the level of the PrV nuclei in some but not all mice stimulated for a set of whiskers (Welker et al., 1992), demonstrating that experience-dependent plasticity could affect subcortical stations in adults. Although even in this case, the expression of subcortical plasticity may depend on cortical feedback (Welker et al., 1988; Krupa et al., 1999).

Enhanced inhibition within the stimulated barrel. A previous study from our laboratory showed that chronic stimulation induces an increase synaptic density in the stimulated barrel (Knott et al., 2002). Both the number of excitatory and inhibitory synapses increase but the proportional increase in inhibitory synapses is robustly higher. The ratio between excitatory and inhibitory synapses shifts from 4.59 in non-stimulated to 2.89 in stimulated animals. Most new inhibitory synapses occur on spines. All spines that bear an inhibitory synapse always possess an excitatory synapse, forming so-called double-synapses spines (Micheva and Beaulieu, 1995; Knott et al., 2002). Following chronic stimulation, the insertion of new inhibitory synapses on spines, either pre-existing or newly formed, leads to a nearly 4 fold increase in the number of double-synapses spines (Knott et al., 2002). Thus, the experience-dependent depression of PW-evoked firing during T2 in the stimulated barrel is likely a functional consequence of the increased density of inhibitory synapses at the level of layer IV: activation of inhibitory neurons by the TC-volley and subsequently by excitatory layer IV neurons could engage stronger feedforward and feedback inhibition via the activation of $GABA_A$ and $GABA_B$ postsynaptic receptors (Barnard et al, 1998; Bowery et al, 2002 for a review).

Additionally, GABA release from inhibitory synapses can escape from the synaptic cleft and activates $GABA_B$ presynaptic receptors on neighbouring, excitatory synapses, depressing the glutamate release (Isaacson et al., 1993; Gil et al., 1997). Presynaptic effects of GABA may be of importance in the context of the experience-dependent depression of neuronal spiking observed in the present study because, being prominent on spines, the newly formed inhibitory synapses will be in close proximity with excitatory synapses. In agreement with the assumption that chronic stimulation does

not affect transmission at TC-synapses, it has been shown that presynaptic $GABA_B$ mediated inhibition affects IC- rather than TC-excitatory synapses, presumably because of the lack of presynaptic $GABA_B$ receptors onto TC-axon terminals (Dehay et al., 1991; Gil et al., 1997). However, contradictory results were recently reported by Porter and Nieves (2004).

It was shown *in vitro* that GABAergic synapses can undergo bidirectional long term changes in synaptic strength following repetitive presynaptic stimulation in different brain regions (Marty and Llano, 1995; Woodin et al., 2003). Changes in synaptic strength at pre-existing GABAergic synapses and structural synaptic plasticity are not mutually exclusive, the former could occur rapidly after the beginning of the chronic stimulation protocol while structural rearrangement of synapses could develop later.

Decrease in spontaneous activity. Along with the depression of PW-evoked responses, spontaneous activity is also decreased in the stimulated barrel. During urethane anesthesia, both excitatory and inhibitory neurons in the neocortex exhibit spontaneous bursts of APs and/or changes in Vm that can occur synchronously in neighbouring neurons (Steriade et al., 1993; Arieli et al., 1995; Lampl et al., 1999). It has been reported in the somatosensory cortex of rodents that spontaneous activity is correlated across neurons in areas encompassing several barrel columns and that the strength of this correlation depends on the distance between recorded neurons (Armstrong-James et al., 1985; Erchova et al., 2002; Petersen et al., 2003b). Petersen and colleagues have demonstrated that in layers II&III spontaneous activity either propagates as waves of excitation across the somatosensory cortex or remain localized to a single barrel column. Moreover, spontaneous activity likely depends on recurrent excitation and inhibition in the cortical network because glutamate receptor blockade suppresses spontaneous firing while GABA receptor blockade enhances it (Armstrong-James et al., 1985; Petersen et al., 2003b). Those observations indicate that the synchronicity of spontaneous activity among nearby neurons likely rely on the network of connections in which neurons are embedded. Therefore it could be hypothesized that the decrease in the ratio of excitatory/inhibitory synapses influences the propagation of spontaneous activity within the stimulated barrel, decreasing spontaneous firing.

A reduction of spontaneous firing might also be due to changes in intrinsic properties of layer IV neurons in the stimulated barrel. Modifications in the chemical environment of neurons might induce a decrease in excitability reducing the probability of spontaneous spikes but not preventing spiking driven by evoked activity. For instance, it is possible that the concentration of extracellular GABA has been upregulated through stronger release at inhibitory synapses, enhanced AP-independent release or modulation of GABA re-uptake (Cavelier et al., 2005). An elevated concentration of extracellular GABA could persist in the stimulated barrel during the recording session we have realized immediately after the 24h period of chronic stimulation. The effect of an increase concentration of extracellular GABA onto the activity of a neuronal population are difficult to predict, however It could modulate

neuronal firing by enhancing tonic inhibitory currents via high affinity extrasynaptic $GABA_A$ receptors (Fritschy et al., 1992; Rossi and Hamann, 1998; Banks and Pearce, 2000; Semyanov et al., 2003). Chronic stimulation may also lead to a persistent reduction of the concentration of glutamate in the extracellular medium, as suggested by the upregulation of glutamate transporters GLAST and GLT-1 in the stimulated barrel (see below).

Decreased excitation at cortico-cortical synapses within the stimulated barrel. Alternatively to the increased inhibition, the reduction of neuronal activity may rely on a decreased excitation in the stimulated barrel. Experience-dependent modification of excitatory synapses between spiny stellate neurons could lead to a lowering of feed-forward amplification. Egger and colleagues (1999) have induced long term depression (LTD) of connections between spiny stellate neurons in slices preparation of the young (P14) rat barrel cortex. By pairing APs in presynaptic neurons with postsynaptic neurons APs during short 10 Hz trains, they found a depression of the EPSPs amplitude that lasts at least one hour following the pairing protocol and that depends on the modulation of group II metabotropic glutamate receptors of the presynaptic membrane. However, to our knowledge, LTD at layer IV synapses has not been reported in adult animals so far. Evoked spiking generated by cortico-cortical excitatory synapses crucially depends on postsynaptic NMDA-Rs activation (Armstrong-James et al., 1993; Fleidervish et al., 1998). Therefore, experience-dependent downregulation of those receptors, or modifications in their subunit expression, may also constitute a potential mechanism for reduced firing during T2, i.e. by reducing EPSP amplitude below the AP threshold within the layer IV excitatory network. Moreover, besides their function in neurotransmission, NMDA-Rs are key actors in the induction of experience-dependent plasticity (Rema et al., 1998) because of their slow kinetics that confer them the capacity to act as coincidence detectors and because they mediate postsynaptic Ca^{++} influx which is necessary for changes in synaptic strength (Cull-Candy and Leszkiewicz, 2004 for a review).

II.4. Depression of PW-evoked single unit responses in layers II&III

Intra-columnar relay of sensory signals through layer IV-layers II&III projections. PW-evoked responses in layers II&III neurons of the C2-barrel column show a delay in mean modal latency of ~ 3-5 ms as compared to layer IV neurons in both non-stimulated and stimulated mice (10-11 ms in layer IV versus 14-15 ms in layers II&III). These latency differences between earliest discharges in the two layers reflect a critical feature of sensory processing: following their activation by TC-afferents, layer IV neurons relay sensory signals to layers II&III in a columnar fashion (Armstrong-James et al., 1992). It has been shown that the EPSP onset evoked by thalamic stimulation or PW-deflection is faster in neurons of layer IV than of layers II&III in the same barrel column (Agmon and Connors, 1992; Carvell and Simons, 1988; Moore and Nelson, 1998; Zhu and Connors, 1999; Wilent and Contreras, 2004),

which support the notion that longer latency of AP firing in layers II&III is due to a synaptic relay of evoked activity from layer IV to layers II&III. Using a voltage sensitive dye, optical imaging studies in slice preparations from the rat brain have demonstrated that the stimulation of a single barrel generate an excitatory signal that is initially restricted to the stimulated barrel and then spread in a columnar manner into layers II&III (Petersen and Sakmann, 2001; Laaris and Keller, 2002). In agreement with that, the axonal projections of layer IV excitatory neurons in layers II&III are confined to the width of their home-barrel column. In layers II&III, these axons contact pyramidal neurons, whose dendritic arborizations area is similarly restricted to the border of the barrel column, thus providing an anatomical substrate for a columnar propagation of excitation (Harris and Woolsey, 1983; Simons and Woolsey, 1984; Gottlieb and Keller, 1997; Lubke et al., 2000; Feldmeyer et al., 2002; Petersen et al., 2003a).

Nearly all supratreshold responses in layers II&III of the rat barrel cortex requires the activation of NMDA-Rs (Armstrong-James et al., 1993). Intracellular recordings in slice preparations demonstrate a strong coupling between layer IV and layers II&III excitatory neurons in the same column (Feldmeyer et al., 2002). Both AMPA and NMDA-Rs contribute to the EPSPs generated by layer IV neurons at the soma of layers II&III pyramidal neurons (Feldman et al., 1998; Feldmeyer et al., 2002). The somatic EPSPs evoked by a layer IV neuron in a layers II&III neuron are reliable but on average of lower amplitude than those evoked in a neighbouring layer IV neuron (Feldmeyer et al., 2002; Silver et al., 2003). These observations have suggested that the connections between layer IV and layers II&III neurons efficiently transmit excitation and could act as an activity-dependent gate, requiring high level of layer IV input excitation to propagate activity in layers II&III. Porter et al. (2001) found a preponderance of layer IV inhibitory interneurons axonal projections directed to layers II&III, suggesting that after thalamocortical input, a vertical flow of inhibition follows excitation along its intracortical course toward the upper layers (Wilent and Contreras, 2004).

Transient Downregulation of neuronal responses in layers II&III of the stimulated barrel column. Following chronic C2-whisker stimulation, mean PW-evoked RM of neurons in layers II&III of the corresponding barrel column decreases by 36% as compared to PW-evoked RM in the same barrel column of non-stimulated animals. This depression of PW-evoked response is restricted to the stimulated barrel column as the response of neurons in adjacent barrel columns C1 and D2 towards their own PW are not altered following chronic stimulation (see figure 16). Similarly, layers II&III neurons spontaneous activity is depressed only in the stimulated barrel column. These effects are transient as both spontaneous and evoked neuronal activities were no longer depressed 4 days after the stimulation.

After the deflection of the PW, layers II&III neurons already fire spikes during the time epoch T1, driven by TC-axons that provide some inputs to layers II&III and

possibly by earliest spikes from layer IV neurons. In non-stimulated animals, mean neuronal firing frequency of layers II&III neurons during T2 rises above the level sustain during T1, presumably reflecting the transmission of layer IV activation to layers II&III during T2. Following chronic stimulation, PW-evoked firing during T2 does not exceed the level reached during T1 in the stimulated barrel column. Quantitative analysis reveals that chronic stimulation robustly decreases mean neuronal firing during the T2 and the subsequent T3 and T4 epochs of the response while the earliest epoch is not altered. Thus, given the depression of neuronal responses in layer IV following chronic stimulation, the decrease response in layers II&III is likely due to a decrease in the vertical flow of excitation to layers II&III from layer IV neurons.

The reduction of response magnitude in layers II&III is even stronger than that in layer IV (33% vs 25%). This stronger decrease of layers II&III firing could reflect the fact that a greater proportion of evoked firing in layer IV are generated by the TC-volley which was not altered after chronic stimulation. Another possibility is that the gain control of the layers II&III spike output could amplify the decrease of layer IV evoked activity. A reduction in the output from layer IV neurons may lead to a disproportional decrease in suprathreshold responses in layers II&III neurons. Alternatively, the strength of layer IV to layers II&III synapses could have been weakened through a LTD-like process in the stimulated barrel column (Finnerty et al., 1999; Feldman, 2000). Finally one could hypothesize an increase in inhibition transmitted to layers II&III from layer IV inhibitory neurons (Porter et al, 2001). Such an experience-dependent increase in inhibition in layers II&III is supported by the increase in GAD immunoreactivity following four days of whisker stimulation which is maximum in layer IV but also invade layers II&III (Welker et al., 1989a). Yet, we do not know whether structural changes do occur at the level of the inhibitory synaptic network in layers II&III after chronic stimulation.

Short-term dynamics of neuronal responses in layers II&III. As in layer IV, RM of layers II&III neurons significantly decreases with incremented frequency of PW-deflections between 0.5 and 8 Hz in both non-stimulated and stimulated mice. Chronic stimulation depresses RM, however the decrease in RM does not reach significance when the PW was deflected at 8 Hz (Tukey's tests). Mean RM to trains of whisker deflection applied at 8 Hz is 4.4 and 3.1 fold lower than to 0.5 Hz trains in non-stimulated (0.71 vs 0.16) and stimulated (0.41 vs 0.14) animals respectively. As compared to the adaptation of layer IV neurons, the adaptation of neuronal responses seems to be stronger in layers II&III than in layer IV where we observe a 2 fold decrease of RM between 0.5 and 8 Hz, which could reflect additional depression at layer IV - II&III synapses. Short-term synaptic depression has often been attributed to a decrease release of neurotransmitters however it could results from both presynaptic and postsynaptic mechanisms. For instance, postsynaptic receptors composition may influence the response to trains of stimulus in a frequency-dependent manner. Long decay time of NMDA currents at layer IV - II&III synapses could maintain the

membrane potential in layers II&III neurons at a more depolarized level than resting potential, reducing the driving force for subsequent glutamatergic excitation at layer IV - II&III synapses.

The GLM regression analysis reveals that the frequency effect onto mean RM of layers II&III neurons is not equivalent in non-stimulated and stimulated animals. The correlation coefficient characterizing the slope of the regression line of the relationship between frequency and RM is lowered after chronic stimulation (t-tests), indicating that adaptation in layers II&III could be stronger in non-stimulated mice than in stimulated mice. This reduction of adaptation after chronic stimulation may reflects the fact that stimulus-evoked responses, even to the first stimulus, are already small in stimulated animals.

III. Single unit responses in “non-stimulated” barrel columns

Neurons in a barrel column respond to several whiskers at the subthreshold and supratreshold levels, the PW yielding the strongest and fastest responses (Welker, 1976; Armstrong-James and Fox, 1987; Welker et al., 1993; Moore and Nelson, 1998; Zhu and Connors, 1999). We then ask the question as to whether neuronal responses to the chronically stimulated C2-whisker were also modified in barrel columns neighbouring the C2-barrel columns, i.e. non-stimulated barrel columns. The present study shows that upon the deflection of the C2-whisker, neurons of the C2-barrel column fire earlier, and more robustly, than neurons in neighbouring barrel columns C1 and D2 (see Table 2 and 3). Based on latency analysis of neuronal responses throughout the barrel field of rats, it has been proposed that the pathways from SW and PW to neurons within a barrel column were different and that the SRF of neurons was dependent on intracortical inter-barrel column connections (Armstrong-James et al., 1992). Following this model, PW-evoked excitation first invades the corresponding barrel then engages the entire column and subsequently radiate out into the neighbouring barrel columns. In correspondence with that, the responses of neurons in a barrel column to a given SW crucially depend on the integrity of neuronal responses in the barrel column corresponding to this whisker (Armstrong-James et al., 1991; Fox, 1994; Fox et al., 2003).

Anatomical studies support the existence of an intracortical input for SW responses that could participate to the SRF in layers II&III: long-range horizontal projections of layers II&III axons span several barrel columns (Gottlieb and Keller, 1997; Lubke et al., 2003; Petersen et al., 2003a), a small proportion of collaterals of axonal projections from layer IV neurons to supragranular and infragranular layers "fan out" to adjacent barrel columns (Lubke et al., 2000) and collaterals of the vertical projections between the supragranular and infragranular layers often terminate in adjacent barrel columns (Bernardo et al., 1990b; Chagnac-Amitai et al., 1990). The origin of SW-responses in layer IV is controversial (Goldreich et al., 1999). SW responses could also theoretically

originates from TC-cells in the corresponding barreloid that would transmit their SRF to the cortex (Simons and Carvell, 1989; Brecht and Sakmann, 2002b) or from somewhat divergence of TC projections form VB in several cortical barrels (Land et al., 1995; Keller and Carlson, 1999; Arnold et al., 2001; Gheorghita-Baechler and Welker, 2005). Optical imaging studies in slice preparations have shown that activity evoked in a barrel column does not spread directly to an adjacent barrel in layer IV (Petersen and Sakmann, 2001; Laaris and Keller, 2002), suggesting that barrels could be considered to function independently of one another. This assumption is reinforced by the rarity of known horizontal cortico-cortical connections between neurons of the barrel hollows, although they exist, in both rats and mice (Bernardo et al., 1990a; Lubke et al., 2000; Petersen and Sakmann, 2000; Brecht and Sakmann, 2002a). However, apical dendrites of some cells in layer IV should sample the activity of the horizontal projections in layers II&III (Simons and Woolsey, 1984; Lubke et al., 2000; Brecht and Sakmann, 2002a; Staiger et al., 2004; Valverde, 1986; Genoud et al., 2004). In addition, recent studies have demonstrated the existence of functional direct connections between barrels in layer IV (Schubert et al., 2003; Staiger et al., 2004). Thus, it is possible that subcortical and cortical pathways cooperate to the construction of the SRF in the barrel (Petersen and Diamond, 2000; Petersen et al., 2003a).

As illustrated in figure 16, C2-whisker responses are decreased in layers II&III of the C1- and D2-barrel columns but not in layer IV, following chronic whisker stimulation. We proposed that the decrease of neuronal responses in layers II&III is a direct consequence of the depression of the responses evoked by the chronically stimulated whisker in the corresponding barrel column, which likely should reduced the level of inter-barrel column excitation. Unaltered responses in layer IV suggest that a significant proportion of the spikes evoked by the chronically stimulated whisker in the C1- and D2-barrels are driven by a different pathway than those in layers II&III. In agreement with that, layers II&III transcolumnar projections are thought to largely avoid layer IV (Lubke et al., 2000; Petersen et al., 2003a; Brecht et al., 2003). A likely route for the relay of C2-responses to adjacent barrels is layer VI collaterals (Staiger et al., 1996b; Gottlieb and Keller, 1997; Zhang and Deschenes, 1997). Another possibility is that C2-whisker responses in the C1- and D2-barrel are at least partially mediated by divergent TC connections. Chronic stimulation may have not altered transmission in these pathways.

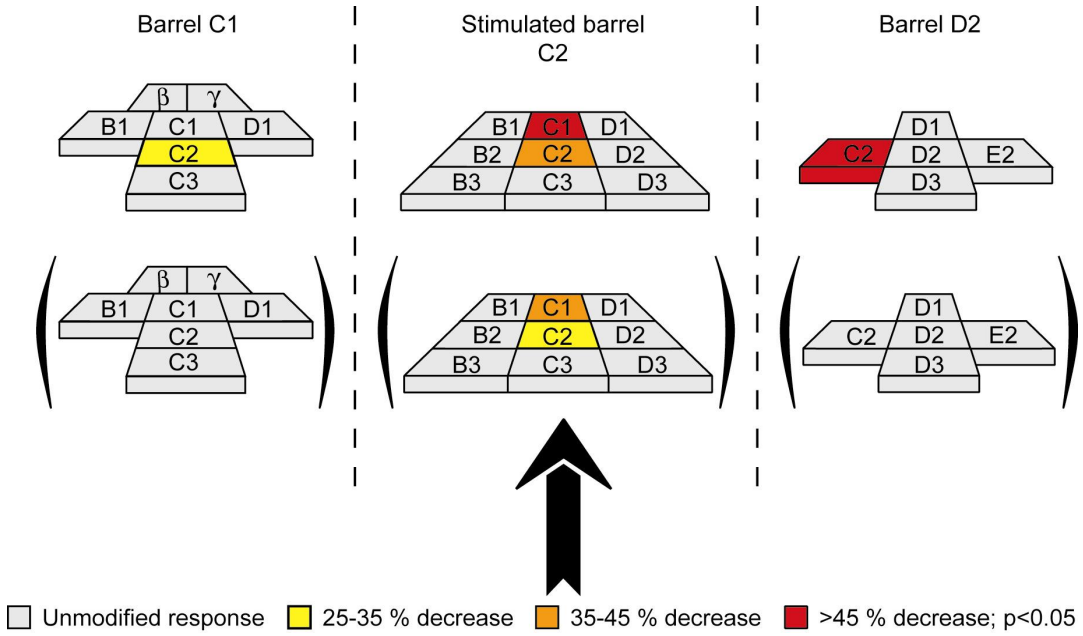


Figure 16. Experience-dependent changes within the receptive field of cortical neurons
Experience-dependent changes within the receptive field of cortical neurons in layer IV, indicated by the schematic barrel borders, and in the above layers II&III of the C2-barrel column and in adjacent barrel columns C1 and D2 following 24h of C2-whisker stimulation. Squares colour coding represents the percentage change in mean response magnitude between the neuronal populations of the non-stimulated and the 24h stimulated animals towards each whisker, whose identity is indicated in each square. Upon the deflection of a whisker, cortical processing is initiated in the corresponding barrel column at the level of the layer IV, which is the main recipient of TC-inputs from the VB nuclei. This is illustrated by the arrow for whisker C2 responses. Layer IV neurons relay evoked activity to layers II&III in a columnar manner. The flow of excitation then spread in neighbouring barrel columns (Armstrong-James et al, 1992), presumably through trans-barrel columns projections of layers II&III neurons. Responses to SWs in layer IV however should also depend on direct TC connections (see text). Following chronic whisker stimulation, response magnitude of neurons towards the stimulated whisker is decreased in layer IV and in layers II&III of the C2-barrel column, i.e. the stimulated barrel column, and in layers II&III but not layer IV of adjacent non-stimulated barrel columns C1 and D2. Responses towards the C1-whisker are unchanged in the corresponding barrel column but are strongly decreased in the stimulated barrel. Responses to all other whiskers are not affected by the chronic stimulation in the stimulated and non-stimulated barrel columns.

B. Alteration within the surround receptive field of neurons in the stimulated barrel column

Not all responses in the stimulated barrel column are depressed following chronic stimulation. Within the SRF of neurons in the C2-barrel column, responses towards SWs that are not in row C, i.e. Arc-1, Arc-2 and Arc-3 whiskers, are not altered (see figure 16). However, responses towards the C1-whisker are significantly depressed after chronic stimulation. The depression of neuronal firing alters the entire duration of

the C1-evoked response in both layer IV and layers II&III. Responses to the deflection of the C3-whisker are also lower, although not significantly, following chronic stimulation.

Given that PW-responses in barrel column C1 are not reduced after the chronic stimulation of the C2-whisker, the strong decrease of the C1-evoked responses in the C2-barrel column should be due to a mechanism that takes place within the C2-barrel column itself or in the relay between C1 and C2 barrel columns. Since not all SW-responses are depressed, the depression is not the result of a global dampening of neuronal responsiveness in the stimulated barrel column, but rather from mechanisms that specifically alter some but not all synaptic inputs to the stimulated barrel column in function of previous experience. Unlike during whisking behaviours in which neighbouring whiskers are often simultaneously activated, during chronic stimulation activity in the stimulated pathway is not correlated to the activity in the pathways from intact whiskers. This chronic "uncoupling" may drive depression at synaptic inputs in the stimulated barrel column from SWs following Hebbian-like rules of plasticity (Hebb, 1949; Cruikshank and Weinberger, 1996; Martin et al., 2000). Among the SWs, in-row whiskers evoke the strongest and fastest responses in barrel column C2. This anisotropy upon latencies and RM for in-row whiskers and the other SWs were already observed in both rats and mice (Simons, 1978; Welker et al., 1993) and relies on the preferred propagation of evoked excitation along the rows of the barrel columns, mainly through horizontal axonal projections of layers II&III neurons (Petersen et al., 2003a). The activity evoked at the specific synaptic inputs from the in-row whiskers, whether originating form inter-barrel projections or direct TC-projections, could have reached a threshold for an Hebbian-like mechanism of plasticity during the prolonged period of whisker stimulation. In correspondence with this assumption, previous studies have proposed that modifications of effective intracortical connectivity following Hebbian-like rules could account for the plasticity of adult sensory cortex *in vivo* (Armstrong-James et al., 1994; Fox, 1994; Wang et al., 1995).

The depression of neuronal responses towards the C2-whisker and the C1-whisker may not result from the same mechanism. One mechanism that takes place within the stimulated barrel depresses responses to the chronically stimulated whisker, presumably by means of an enhanced feedforward inhibition mediated by inhibitory interneurons. In this context, it is reasonable to think that those inhibitory interneurons preferentially modulate excitation generated by the chronically stimulated PW. Another mechanism specifically alters responses to in-row whiskers by modifying their transcolumnar synaptic inputs to the stimulated barrel column following Hebbian-like rules synaptic depression.

Alternatively, it is possible that our measurements of neuronal activity were not sensitive enough to detect significant changes at the low level of neuronal firing evoked by SWs other than C1 and that unspecific depression alters all responses in the stimulated barrel column, for instance through a disequilibrium between excitation and

inhibition. Indeed, SWs deflections evoke spike counts that are often just above our limit of approval for a significant response (<0.1 spike per deflection above spontaneous activity) and the level of responses to a particular SW is highly variable across cells. However, we do not observe a general tendency towards lower level of mean RM to SWs from Arc-1 to -3, and the number of SWs eliciting activity above spontaneous level per neuron is not altered after chronic stimulation (see Table 2), which would have been unlikely if chronic stimulation had reduced the responses to weak inputs from SWs.

C. The post inhibitory rebound: long term effect of chronic stimulation

Four days after the stimulation was stopped, spontaneous activity of neurons in the stimulated barrel column is returned to control level. Responses of neurons towards the chronically stimulated whisker as well as towards in-row whiskers are no longer depressed. On the contrary, response magnitude of neuronal responses towards the chronically stimulated whisker is slightly but significantly increased in layer IV of the corresponding barrel column, which indicates that the chronic stimulation has a long term effect onto cortical processing. The epoch analysis reveals a significant increase of the second component of AP spiking between 50-100 ms (T4) after the PW-deflection.

The second component of neuronal spiking in layer IV has often been referred as a post inhibitory rebound (PIR) and is dependent on the level of anesthesia or the behavioural state (Simons et al., 1992; Fanselow and Nicolelis, 1999). The PIR reflects an intrinsic property of neurons in many parts of the brain, including the cerebral cortex. Hyperpolarization of neurons, by current injection or recruitment of local inhibition after afferent activation, is followed by a rebound depolarization that can be strong enough to increase spiking probability (Stafstrom et al., 1984; Huguenard, 1996; Grenier et al., 1998). The rebound amplitude is positively correlated to the degree of the preceding hyperpolarization (Stafstrom et al., 1984; Dean et al., 1989; Steriade and Timofeev, 1997; Fan et al., 2000). Morphologically, 4 days after the cessation of the stimulation, the total synaptic density in the stimulated barrel regains the level measured in non-stimulated animals; however, the density of GABAergic synapses on spines remains equivalent as in animals analysed immediately after the stimulation, i.e. at highest level than in non-stimulated animals (Knott et al., 2002). It is tempting to speculate that the increase in rebound activity four days after the stimulation may be related to the maintenance of GABAergic synapses on spines. One could hypothesize that a modified equilibrium between excitatory and inhibitory synapses amplifies the level of hyperpolarization that follows the activation of layer IV neurons and in turn potentiates the magnitude of the PIR. However, since our extracellular single unit recordings only gives information onto suprathreshold activity,

we do not know how the subthreshold membrane potentials actually evolve and how the underlying EPSPs and IPSPs have been affected by the chronic stimulation.

Thus, four days after the cessation of the stimulation, experience-dependent modifications are still present at the morphological and functional level. Although it is difficult to establish the link between morphology and neuronal function, the long-lasting morphological and functional changes indicate that the sensory experience has left a persistent mnesic trace in layer IV of the primary somatosensory cortex. At present, we do not know the exact function of GABAergic synapses on spines, nor of a stronger PIR in layer IV. PIR has been implicated in various processes such as brain rhythms or augmenting response (Grenier et al., 1998). Interestingly, the dynamic of the PIR in mice establishes a periodicity that matches the whisking frequency range (5-15 Hz; Welker, 1964), which may allow an ideal temporal frame for the extraction of spatiotemporal clues during exploratory behaviours. Spikes that are not stimulus locked should be specifically suppressed by the long-lasting inhibition that is thought to precede the second component of AP spiking. Four days after chronic stimulation, depression of evoked responses is no longer present; however, the increased magnitude of the second component of evoked firing during 50-100 ms poststimulus may represent a long-term effect of a prolonged period of sensory experience during which trains of passive whisker stimulus were separated by ~ 60 ms.

D. Upregulation of glutamate transporters following whisker stimulation

The present study provides evidence that astrocytes are active partners during experience-dependent plasticity in the adult cerebral cortex. The chronic stimulation induces an increase in the expression of GLAST and GLT-1 proteins in the stimulated barrel column. This up-regulation is transient as the level of expression of both proteins return to control levels, 4 days after the stimulation was halted.

GLAST and GLT-1 are the two astrocytic glutamate transporters that are responsible for almost all the removal of extracellular glutamate in the mammalian cortex (Tanaka et al., 1997; Rothstein et al., 1996). As glutamate is not metabolized in the extracellular environment, the regulation of the level of glutamate outside cells strongly depends on cellular uptake by these active transporters proteins located in the plasma membrane of astrocytes (Danbolt, 2001). Studies in GLT-1 knock-out mice have shown a higher peak concentration and a longer elevated concentration of glutamate in the extracellular environment after its synaptically release (Tanaka et al., 1997). It was already shown that glutamate transporter expression could be modulated by neuronal soluble factors, such as glutamate itself *in vitro* (Gegelashvili et al., 2001; Gegelashvili et al., 1996; Gegelashvili and Schousboe, 1997), or by synaptic activity in primary hippocampal cultures (Perego et al., 2000). Levy and colleagues (1995; 1993b) also observed reduction in the two astrocytic glutamate transporters in the

striatum of the rat after corticostriatal nerve terminal degeneration. However, the present increase in the expression of GLAST and GLT-1 is, to our knowledge, the first *in vivo* evidence showing that the level of expression of these transporters in the adult cortex is regulated in an activity-dependent manner upon innocuous changes in sensory experience.

Stronger expression of glutamate transporters may increase the transport ability of astrocytes and enhanced their glutamate clearance function. During whisker stimulation, glutamate is continuously released by TC-afferents and excitatory synapses of cortical neurons (Agmon and Odowd, 1992; Armstrong-James et al., 1993) in the corresponding barrel column; at the end of the stimulation period neurons still demonstrate excitatory evoked responses towards PW-deflections applied at frequencies similar to that driving the passive movement of the whisker in the stimulator (~9 Hz).

In the context of this chronic release, the control of glutamate level in the extracellular medium appears to be primordial for several reasons. First, the level of the extracellular concentration of glutamate will determine the extent of receptor stimulation after its release in the synaptic cleft. A low extracellular concentration is required for a high signal-to-noise ratio in synaptic and extrasynaptic transmission since synaptic clefts are in continuity with the extracellular space. Moreover, glutamate transporters may play a key function in neurotransmission itself. *In vitro* blocking of glutamate transport potentiates postsynaptic excitation (Mennerick and Zorumski, 1994). Active translocation through transporters affect glutamate concentration in the synaptic cleft on a milliseconds timescale and influence time-course of synaptic transmission by curtail excitatory postsynaptic current (Diamond and Jahr, 1997; Rauen et al., 2000; Grewer et al., 2000; Takahashi and Attwell, 1995; Overstreet et al., 1999). In correspondence with that, at a subcellular level, Lehre and colleagues have observed that GLAST and GLT-1 are preferentially localized in astroglial membranes facing dendritic spines and axons than in membranes facing other astrocytes or cell bodies (Lehre et al., 1993; Chaudhry et al., 1995), suggesting these transporters could be preferentially localized near synapses. Besides this synaptic function, extracellular concentrations of glutamate in brain must also be strictly maintained at non toxic levels (Tanaka et al., 1997; Watanabe et al., 1999).

Therefore, in the context of a prolonged period of chronic whisker stimulation, we hypothesize that glutamate transporter upregulation could be fundamental for limit excitation spread and perturbation of synaptic cross-talk. One can imagine that after the cessation of the stimulation, the increased glutamate clearance function could keep tonic glutamate concentrations in the extracellular space low, contributing to the reduction of spontaneous firing in the "stimulated" barrel column. Moreover, an increased glutamate capture and transport may support the decreased in evoked firing in the cortical column by altering the glutamate neurotransmission.

These observations are strengthened by the results of another study in our laboratory that demonstrated an increase glial coverage of synaptic complex following chronic stimulation (Quairiaux et al., 2005). Experience-dependent morphological changes at the level of astrocytes may participate to the mechanism that control the glutamate release (Oliet et al., 2001), by building barriers to glutamate over-spill from the synapses and bringing new transporters near the location of glutamate release.

CONCLUSIONS AND FURTHER INVESTIGATIONS

Using selective whisker deprivation in adult rodents, it has been shown that the preferred usage of a single whisker, or a pair of whiskers can lead to a potentiation of neuronal responses after 24h in layers II&III and after 2-3 days in layer IV of the corresponding barrel columns (Diamond et al., 1994; Armstrong-James et al., 1994; Barth et al., 2000). The present study reports a new form of experience-dependent plasticity in the adult barrel cortex: following a 24h period of increased stimulation of a single whisker, the magnitude of neuronal response towards this whisker is depressed in both layer IV and layers II&III of the corresponding barrel column. The mouse passively receives the increased stimulation therefore depression of cortical responses to the stimulated whisker that we observe at the end of the stimulation period may represent a habituation-like phenomenon to a continuous and behaviourally irrelevant stimulus.

The depression of evoked responses does not appear to be due to a decrease in the activation of the barrel column by its TC-afferents but rather relies on a mechanism of cortical origin. The enhanced density of GABAergic synapses in layer IV indicates that this mechanism could be mediated by inhibitory interneurons (Knott et al., 2002). In response to chronic stimulation, new GABAergic synapses are inserted on dendritic spines of layer IV excitatory neurons, which could enhance feedforward inhibition evoked by the TC-volley and account for the decrease in neuronal firing during a corticocortical step of sensory processing in layer IV. A lower level of evoked firing in a barrel should lead to a weaker propagation of excitation within the column. Accordingly, the depression of evoked firing in layers II&III of the stimulated barrel column, and of neighbouring barrel columns, may result from a decreased flow of excitation evoked in the intercolumnar and transcolumnar pathways. Several studies have shown that experience-dependent plasticity develops faster and has a stronger impact in layers II&III than in layer IV (Buonomano and Merzenich, 1998). In this context, further work would be needed to establish whether modifications of neuronal responses appear first in layer IV or in layers II&III or simultaneously by performing recordings after shorter periods of stimulation. Furthermore, it would be necessary to investigate whether chronic stimulation also alters synaptic structure in layers II&III.

We have no direct evidence, however, that an increased inhibition in the cortical column is responsible for the decreased neuronal firing. One indirect approximation of inhibition within the stimulated barrel could be the study of paired-pulse depression with inter-stimulus intervals of 10-50 ms. The precise mechanisms by which response

properties of neurons are modified with experience are difficult to infer with suprathreshold recording techniques. *In vivo* intracellular recordings would be necessary to determine the dynamic and the relative amplitude of spontaneous and evoked excitatory and inhibitory currents as well as intrinsic neuronal properties such as resting potentials in layer IV spiny and aspiny neurons following chronic stimulation.

While their PW-responses are depressed, neurons of the stimulated barrel column show unaltered responses towards all SWs with the exception of in-row whiskers. Two different forms of experience-dependent plasticity may alter differentially neuronal responses evoked by the PW and by the whiskers in the SRF. First, enhanced inhibition within the stimulated barrel may specifically modulate the magnitude of responses generated by the direct TC-afferents from the stimulated pathway, i.e. PW-responses. Second, the prolonged period of uncorrelated activity between the pathways from the stimulated whisker and from intact whiskers may specifically weaken the synaptic inputs from in-row SWs.

Depression of neuronal responses was no longer present 4 days after the cessation of chronic stimulation. On the contrary, responses to the PW appear to be potentiated, with a significant increase in the magnitude of the second component of evoked firing. Therefore, although the modifications of neuronal responses observed immediately after the stimulation period are transient, chronic stimulation leaves a long lasting trace in the cortical circuitry. Further investigations should address the duration of these functional alterations. Finally, it raises the interesting questions as to whether, and how, these alterations should influence the experience-dependent adaptations of the cortical network to a second period of chronic stimulation.

Finally, by demonstrating the *in vivo* regulation of astrocytic glutamate transporters expression, we show that activity-dependent structural changes at the synaptic level and modifications of neuronal responses are part of a more general ensemble of adaptative mechanisms in the cortex that include glial cells. The continuous release of glutamate at the level of the cortical network during chronic stimulation could potentially disturb the specificity of neuronal transmission through spill-over and intersynaptic cross-talk (Huang and Bergles, 2004). The up-regulation of glutamate transporters by neuronal activity presumably allows increased potential transport abilities to face increased release and suggests a mechanism by which glial cells could decrease excitability in the neuronal network, in response to overstimulation. Further studies should be dedicated to the precise localization of these transporters at a subcellular level and the activity-dependent regulation of their targeting. Interestingly, the new method of cortical biopsy we have developed for this study, combined to biochemical and molecular biology tools, allows one to study *in vivo* multiple genes or protein modulation in a functionally identified cortical region, as small as one barrel column, in relation to sensory experience.

References

Agmon A, Connors BW (1991) Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. Neuroscience 41: 365-379.

Agmon A, Connors BW (1992) Correlation between intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. Journal of Neuroscience 12: 319-329.

Agmon A, Odowd DK (1992) Nmda receptor-mediated currents are prominent in the thalamocortical synaptic response before maturation of inhibition. Journal of Neurophysiology 68: 345-349.

Agmon A, Yang LT, Jones EG, O'Dowd DK (1995) Topological precision in the thalamic projection to neonatal mouse barrel cortex. Journal of Neuroscience 15: 549-561.

Ahissar E, Sosnik R, Bagdasarian K, Haidarliu S (2001) Temporal frequency of whisker movement. II. Laminar organization of cortical representations. Journal of Neurophysiology 86: 354-367.

Ahissar E, Sosnik R, Haidarliu S (2000) Transformation from temporal to rate coding in a somatosensory thalamocortical pathway. Nature 406: 302-306.

Akhtar ND, Land PW (1991) Activity-dependent regulation of glutamic acid decarboxylase in the rat barrel cortex: effects of neonatal versus adult sensory deprivation. Journal of Comparative Neurology 307: 200-213.

Allen CB, Celikel T, Feldman DE (2003) Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. Nature Neuroscience 6: 291-299.

Angulo MC, Lambolez B, Audinat E, Hestrin S, Rossier J (1997) Subunit composition, kinetic, and permeation properties of AMPA receptors in single neocortical nonpyramidal cells. Journal of Neuroscience 17: 6685-6696.

Antonini A, Stryker MP (1996) Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. Journal of Comparative Neurology 369: 64-82.

Araque A, Carmignoto G, Haydon PG (2001) Dynamic signaling between astrocytes and neurons. Annual Review of Physiology 63: 795-813.

Arieli A, Shoham D, Hildesheim R, Grinvald A (1995) Coherent spatiotemporal patterns of ongoing activity revealed by real-time optical imaging coupled with single-unit recording in the cat visual cortex. Journal of Neurophysiology 73: 2072-2093.

Armstrong-James M., Caan AW, and Fox K (1985) Threshold effects of N-methyl-D-aspartate(NMDA) and 2-amino-5-phosphonovaleric acid (2APV) on the spontaneous activity of neocortical single neurones in the urethane anaesthetised rat. Experimental Brain Research 60, 209-213.

Armstrong-James M, Callahan CA, and Friedman MA (1991). Thalamo-cortical processing of vibrissal information in the rat. I. Intracortical origins of surround but not centre

receptive fields of layer IV neurones in the rat S1 barrel field. The Journal of Comparative Neurology 303, 193-210.

Armstrong-James M. and Callahan CA (1991) Thalamo-cortical processing of vibrissal information in the rat. II. Spatiotemporal convergence in the thalamic ventroposterior medial nucleus (VPm) and its relevance to generation of receptive fields of S1 cortical "barrel" neurones. The journal of comparative neurology 303, 211-224. 1991.

Armstrong-James M, Diamond ME, Ebner FF (1994) An innocuous bias in whisker use in adult-rats modifies receptive-fields of barrel cortex neurons. Journal of Neuroscience 14: 6978-6991.

Armstrong-James M and Fox K (1987) Spatiotemporal divergence and convergence in rat "barrel" cortex. Journal of Comparative Neurology. 263, 265-281.

Armstrong-James M, Fox K (1988) Evidence for a specific role for cortical NMDA receptors in slow-wave sleep. Brain research 451: 189-196.

Armstrong-James M, Fox K, Das-Gupta A (1992) Flow of excitation within rat barrel cortex on striking a single vibrissa. Journal of Neurophysiology 68: 1345-1358.

Armstrong-James M, Fox K, Millar J (1980) A method for etching the tips of carbon-fiber microelectrodes. Journal of Neuroscience Methods 2: 431-432.

Armstrong-James M, George MJ (1988) Influence of anesthesia on spontaneous activity and receptive-field size of single units in rat Sm1 neocortex. Experimental Neurology 99: 369-387.

Armstrong-James M and Millar JA (1979) Carbon fibre microelectrodes. Journal of Neuroscience Methods 1, 279-287.

Armstrong-James M, Welker E, Callahan CA (1993) The contribution of NMDA and Non-NMDA receptors to fast and slow transmission of sensory information in the rat Sl barrel cortex. Journal of Neuroscience 13: 2149-2160.

Arnold PB, Li CX, Waters RS (2001) Thalamocortical arbors extend beyond single cortical barrels: an in vivo intracellular tracing study in rat. Experimental Brain Research 136: 152-168.

Aroniadou-Anderjaska A and Keller A (1995). LTP in the barrel cortex of adult rats. Neuroreport 6, 2297-2300.

Artola A, Singer W (1987) Long-term potentiation and NMDA receptors in rat visual cortex. Nature 330: 649-652.

Banks MI, Pearce RA (2000) Kinetic differences between synaptic and extrasynaptic GABA(A) receptors in CA1 pyramidal cells. Journal of Neuroscience 20: 937-948.

Barbour B, Keller BU, Llano I, Marty A (1994) Prolonged presence of glutamate during excitatory synaptic transmission to cerebellar Purkinje-cells. Neuron 12: 1331-1343.

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ (1998) International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 50: 291-313.

Barth AL, McKenna M, Glazewski S, Hill P, Impey S, Storm D, Fox K (2000) Upregulation of cAMP response element-mediated gene expression during experience-dependent plasticity in adult neocortex. Journal of Neuroscience 20: 4206-4216.

Beierlein M, Fall CP, Rinzel J, Yuste R (2002) Thalamocortical bursts trigger recurrent activity in neocortical networks: layer 4 as a frequency-dependent gate. Journal of Neuroscience 22: 9885-9894.

Beierlein M, Gibson JR, Connors BW (2003) Two dynamically distinct inhibitory networks in layer 4 of the neocortex. Journal of Neurophysiology 90: 2987-3000.

Belford GR, Killackey HP (1979) Vibrissae representation in sub-cortical trigeminal centers of neonatal rat. Journal of Comparative Neurology 183: 305-321.

Belford GR, Killackey HP (1980) The sensitive period in the development of the trigeminal system of the neonatal rat. Journal of Comparative Neurology 193: 335-350.

Bender KJ, Rangel J, Feldman DE (2003) Development of columnar topography in the excitatory layer 4 to layer 2/3 projection in rat barrel cortex. Journal of Neuroscience 23: 8759-8770.

Benshalom G, White EL (1986) Quantification of thalamocortical synapses with spiny stellate neurons in layer-IV of mouse somatosensory cortex. Journal of Comparative Neurology 253: 303-314.

Bernardo KL, Mccasland JS, Woolsey TA (1990a) Local axonal trajectories in mouse barrel cortex. Experimental Brain Research 82: 247-253.

Bernardo KL, Mccasland JS, Woolsey TA, Strominger RN (1990b) Local intralaminar and interlaminar connections in mouse barrel cortex. Journal of Comparative Neurology 291: 231-255.

Bernardo KL, Woolsey TA (1987) Axonal trajectories between mouse somatosensory thalamus and cortex. Journal of Comparative Neurology 258: 542-564.

Bezzi P, Volterra A (2001) A neuron-glia signalling network in the active brain. Current Opinion in Neurobiology 11: 387-394.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity - orientation specificity and binocular interaction in visual-cortex. Journal of Neuroscience 2: 32-48.

Bliss TVP, Lomo T (1973) Long-lasting potentiation of synaptic transmission in dentate area of anesthetized rabbit following stimulation of perforant path. Journal of Physiology-London 232: 331-356.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, Enna SJ (2002) International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid(B) receptors: structure and function. Pharmacol Rev 54: 247-264.

Brecht M, Roth A, Sakmann B (2003) Dynamic receptive fields of reconstructed pyramidal cells in layers 3 and 2 of rat somatosensory barrel cortex. Journal of Physiology-London 553: 243-265.

Brecht M, Sakmann B (2002a) Dynamic representation of whisker deflection by synaptic potentials in spiny stellate and pyramidal cells in the barrels and septa of layer 4 rat somatosensory cortex. Journal of Physiology-London 543: 49-70.

Brecht M, Sakmann B (2002b) Whisker maps of neuronal subclasses of the rat ventral posterior medial thalamus, identified by whole-cell voltage recording and morphological reconstruction. Journal of Physiology-London 538: 495-515.

Brodmann K (1909) Vergleichende lokalisationslehre der grosshirnrinde in ihren prinzipien dargestellt auf grund des zellenbaues. Leipzig: JA Barth.

Bruno RM, Simons DJ (2002) Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. Journal of Neuroscience 22: 10966-10975.

Buonomano DV, Merzenich MM (1998) Cortical plasticity: From synapses to maps. Annual Review of Neuroscience 21: 149-186.

Calford MB (2002) Dynamic representational plasticity in sensory cortex. Neuroscience 111: 709-738.

Calia E, Persico AM, Baldi A, Keller F (1998) BDNF and NT-3 applied in the whisker pad reverse cortical changes after peripheral deafferentation in neonatal rats. European Journal of Neuroscience 10: 3194-3200.

Callaway EM, Katz LC (1991) Effects of binocular deprivation on the development of clustered horizontal connections in cat striate cortex. Proceedings of the National Academy of Sciences of the United States of America 88: 745-749.

Carvell GE, Simons DJ (1988) Membrane-potential changes in rat Smi cortical-neurons evoked by controlled stimulation of mystacial vibrissae. Brain research 448: 186-191.

Carvell GE, Simons DJ (1990) Biometric analyses of vibrissal tactile discrimination in the rat. Journal of Neuroscience 10: 2638-2648.

Casado M, Bendahan A, Zafra F, Danbolt NC, Aragon C, Gimenez C, Kanner BI (1993) Phosphorylation and modulation of brain glutamate transporters by protein-Kinase-C. Journal of Biological Chemistry 268: 27313-27317.

Castro-Alamancos MA, Oldford E (2002) Cortical sensory suppression during arousal is due to the activity-dependent depression of thalamocortical synapses. Journal of Physiology-London 541: 319-331.

Cavelier P, Hamann M, Rossi D, Mobbs P, Attwell D (2005) Tonic excitation and inhibition of neurons: ambient transmitter sources and computational consequences. Prog Biophys Mol Biol 87: 3-16.

Caviness VS, Frost DO (1980) Tangential organization of thalamic projections to the neocortex in the mouse. Journal of Comparative Neurology 194: 335-367.

Celikel T, Szostak VA, Feldman DE (2004) Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. Nature Neuroscience 7: 534-541.

Chagnac-Amitai Y, Luhmann HJ, Prince DA (1990) Burst generating and regular spiking layer 5 pyramidal neurons of rat neocortex have different morphological features. Journal of Comparative Neurology 296: 598-613.

Chance FS, Nelson SB, Abbott LF (1998) Synaptic depression and the temporal response characteristics of V1 cells. Journal of Neuroscience 18: 4785-4799.

Chang FLF, Greenough WT (1982) Lateralized effects of monocular training on dendritic branching in adult split-brain rats. Brain research 232: 283-292.

Chapin JK (1986) Laminar differences in sizes, shapes, and response profiles of cutaneous receptive fields in the rat SI cortex. Experimental Brain Research 62: 549-559.

Chaudhry FA, Lehre KP, Campagne MV, Ottersen OP, Danbolt NC, Stormmathisen J (1995) Glutamate transporters in glial plasma-membranes - Highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron 15: 711-720.

Chen R, Cohen LG, Hallett M (2002) Nervous system reorganization following injury. Neuroscience 111: 761-773.

Chiaia NL, Fish SE, Bauer WR, Figley BA, Eck M, Bennett-Clarke CA, Rhoades RW (1994a) Effects of postnatal blockade of cortical activity with tetrodotoxin upon the development and plasticity of vibrissa-related patterns in the somatosensory cortex of hamsters. Somatosensory and Motor Research 11: 219-228.

Chiaia NL, Fish SE, Bauer WR, Figley BA, Eck M, Bennett-Clarke CA, Rhoades RW (1994b) Effects of postnatal blockage of cortical activity with tetrodotoxin upon lesion-induced reorganization of vibrissae-related patterns in the somatosensory cortex of rat. Brain Research Developmental Brain Research 79: 301-306.

Chino YM, Kaas JH, Smith EL, III, Langston AL, Cheng H (1992) Rapid reorganization of cortical maps in adult cats following restricted deafferentation in retina. Vision Res 32: 789-796.

Chmielowska J, Carvell GE, Simons DJ (1989) Spatial-organization of thalamocortical and corticothalamic projection systems in the rat Smi barrel cortex. Journal of Comparative Neurology 285: 325-338.

Chung S, Li XR, Nelson SB (2002) Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses in vivo. Neuron 34: 437-446.

Clark SA, Allard T, Jenkins WM, Merzenich MM (1988) Receptive fields in the body-surface map in adult cortex defined by temporally correlated inputs. Nature 332: 444-445.

Connors BW, Gutnick MJ (1990) Intrinsic firing patterns of diverse neocortical neurons. Trends in Neurosciences 13: 99-104.

Cowan AI, Stricker C (2004) Functional connectivity in layer IV local excitatory circuits of rat somatosensory cortex. Journal of Neurophysiology 92: 2137-2150.

Crair MC, Malenka RC (1995) A Critical period for long-term potentiation at thalamocortical synapses. Nature 375: 325-328.

Croquelois A, Bronchti G, and Welker E (2005) Cortical origin of functional recovery in the somatosensory cortex of the adult mouse after thalamic lesion. European Journal of Neuroscience in press.

Cruikshank SJ, Weinberger NM (1996) Evidence for the Hebbian hypothesis in experience-dependent physiological plasticity of neocortex: A critical review. Brain Research Reviews 22: 191-228.

Cull-Candy SG, Leszkiewicz DN (2004) Role of distinct NMDA receptor subtypes at central synapses. Sci STKE 2004: re16.

Danbolt NC (2001) Glutamate uptake. Progress in Neurobiology 65: 1-105.

Danbolt NC, Stormmathisen J, Kanner BI (1992) An [Na+ +K+] coupled L-glutamate transporter purified from rat-Brain is located in glial-cell processes. Neuroscience 51: 295-310.

Darian-Smith C, Gilbert CD (1994) Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature 368: 737-740.

Dean JB, Czyzykkrzeska M, Millhorn DE (1989) Experimentally induced postinhibitory rebound in rat nucleus ambiguus is dependent on hyperpolarization parameters and membrane-potential. Neuroscience Research 6: 487-493.

Dehay C, Douglas RJ, Martin KAC, Nelson C (1991) Excitation by geniculocortical synapses is not vetoed at the level of dendritic spines in cat visual-cortex. Journal of Physiology-London 440: 723-734.

Delacour J, Houcine O, Talbi B (1987) Learned changes in the responses of the rat barrel field neurons. Neuroscience 23: 63-71.

Deschenes M, Bourassa J, Pinault D (1994) Corticothalamic Projections from Layer-V Cells in Rat Are Collaterals of Long-Range Corticofugal Axons. Brain research 664: 215-219.

Di S, Baumgartner C, Barth DS (1990) Laminar analysis of extracellular field potentials in rat vibrissa/barrel cortex. Journal of Neurophysiology 63: 832-840.

Diamond JS, Jahr CE (1997) Transporters buffer synaptically released glutamate on a submillisecond time scale. Journal of Neuroscience 17: 4672-4687.

Diamond ME, Armstrong-James M, Ebner FF (1992) Somatic sensory responses in the rostral sector of the posterior group (Pom) and in the ventral posterior medial nucleus (Vpm) of the rat thalamus. Journal of Comparative Neurology 318: 462-476.

Diamond ME, Armstrong-James M, Ebner FF (1993) Experience-dependent plasticity in adult-rat barrel cortex. Proceedings of the National Academy of Sciences of the United States of America 90: 2082-2086.

Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. Science 265: 1885-1888.

Dorfl J (1982) The musculature of the mystacial vibrissae of the white-mouse. Journal of Anatomy 135: 147-154.

Dorfl J (1985) The innervation of the mystacial region of the white-mouse - A topographical study. Journal of Anatomy 142: 173-184.

Douglas RJ, Koch C, Mahowald M, Martin KAC, Suarez HH (1995) Recurrent excitation in neocortical circuits. Science 269: 981-985.

Dykes RW, Landry P, Metherate R, Hicks TP (1984) Functional role of GABA in cat primary somatosensory cortex: shaping receptive fields of cortical neurons. Journal of Neurophysiology 52: 1066-1093.

Egger V, Feldmeyer D, Sakmann B (1999) Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. Nature Neuroscience 2: 1098-1105.

Erchova IA, Lebedev MA, Diamond ME (2002) Somatosensory cortical neuronal population activity across states of anaesthesia. European Journal of Neuroscience 15: 744-752.

Erzurumlu RS, Jhaveri S, Benowitz LI (1990) Transient patterns of Gap-43 expression during the formation of barrels in the rat somatosensory cortex. Journal of Comparative Neurology 292: 443-456.

Fan YP, Horn EM, Waldrop TG (2000) Biophysical characterization of rat caudal hypothalamic neurons: Calcium channel contribution to excitability. Journal of Neurophysiology 84: 2896-2903.

Fanselow EE, Nicolelis MAL (1999) Behavioural modulation of tactile responses in the rat somatosensory system. Journal of Neuroscience 19: 7603-7616.

Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron 27: 45-56.

Feldman DE, Nicoll RA, Malenka RC (1999) Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. Journal of Neurobiology 41: 92-101.

Feldman DE, Nicoll RA, Malenka RC, Isaac JTR (1998) Long-term depression at thalamocortical synapses in developing rat somatosensory cortex. Neuron 21: 347-357.

Feldmeyer D, Egger V, Lubke J, Sakmann B (1999) Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single 'barrel' of developing rat somatosensory cortex. Journal of Physiology-London 521: 169-190.

Feldmeyer D, Lubke J, Silver RA, Sakmann B (2002) Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. Journal of Physiology-London 538: 803-822.

Feldmeyer D, Silver RA, Lubke J, Sakmann B (2000) Physiology of synaptic connections between spiny layer 4 neurones and layer 2/3 pyramidal cells in rat barrel cortex. European Journal of Neuroscience 12: 365.

Finnerty GT, Roberts LSE, Connors BW (1999) Sensory experience modifies the short-term dynamics of neocortical synapses. Nature 400: 367-371.

Fleidervish IA, Binshtok AM, Gutnick MJ (1998) Functionally distinct NMDA receptors mediate horizontal connectivity within layer 4 of mouse barrel cortex. Neuron 21: 1055-1065.

Florence SL, Taub HB, Kaas JH (1998) Large-scale sprouting of cortical connections after peripheral injury in adult macaque monkeys. Science 282: 1117-1121.

Foeller E, Feldman DE (2004) Synaptic basis for developmental plasticity in somatosensory cortex. Current Opinion in Neurobiology 14: 89-95.

Fox K (1992) A Critical Period for Experience-dependent synaptic plasticity in rat barrel cortex. Journal of Neuroscience 12: 1826-1838.

Fox K (1994) The cortical component of experience-dependent synaptic plasticity in the rat barrel cortex. The journal of neuroscience 14: 7665-7679.

Fox K (2002) Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. Neuroscience 111: 799-814.

Fox K, Wright N, Wallace H, Glazewski S (2003) The origin of cortical surround receptive fields studied in the barrel cortex. Journal of Neuroscience 23: 8380-8391.

Fritschy JM, Benke D, Mertens S, Oertel WH, Bachi T, Mohler H (1992) Five subtypes of type A gamma-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proceedings of the National Academy of Sciences of the United States of America 89: 6726-6730.

Frost DO, Caviness VS (1980) Radial organization of thalamic projections to the neocortex in the mouse. Journal of Comparative Neurology 194: 369-393.

Furuta A, Rothstein JD, Martin LJ (1997) Glutamate transporter protein subtypes are expressed differentially during rat CNS development. Journal of Neuroscience 17: 8363-8375.

Gaiarsa JL, Caillard O, Ben Ari Y (2002) Long-term plasticity at GABAergic and glycinergic synapses: mechanisms and functional significance. Trends in Neurosciences 25: 564-570.

Gegelashvili G, Civenni G, Racagni G, Danbolt NC, Schousboe I, Schousboe A (1996) Glutamate receptor agonists up-regulate glutamate transporter GLAST in astrocytes. Neuroreport 8: 261-265.

Gegelashvili G, Danbolt NC, Schousboe A (1997) Neuronal soluble factors differentially regulate the expression of the GLT1 and GLAST glutamate transporters in cultured astroglia. Journal of Neurochemistry 69: 2612-2615.

Gegelashvili G, Dehnes Y, Danbolt NC, Schousboe A (2000) The high-affinity glutamate transporters GLT1, GLAST, and EAAT4 are regulated via different signalling mechanisms. Neurochemistry International 37: 163-170.

Gegelashvili G, Dehnes Y, Danbolt NC, Schousboe A (2001) Neuronal regulation of glutamate transporters: molecular and cellular mechanisms. Journal of Neurochemistry 78: 115.

Gegelashvili G, Schousboe A (1997) High affinity glutamate transporters: Regulation of expression and activity. Molecular Pharmacology 52: 6-15.

Genoud C, Knott GW, Sakata K, Lu B, Welker E (2004) Altered synapse formation in the adult somatosensory cortex of brain-derived neurotrophic factor heterozygote mice. Journal of Neuroscience 24: 2394-2400.

Gheorghita-Baechler F and Welker E (2005) unpublished results.

Gibson JM, Welker WI (1983) Quantitative studies of stimulus coding in first-order vibrissa afferents of rats. 1. Receptive field properties and threshold distributions. Somatosensory Research 1: 51-67.

Gibson JR, Beierlein M, Connors BW (1999) Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402: 75-79.

Gil Z, Amitai Y (1996) Adult thalamocortical transmission involves both NMDA and non-NMDA receptors. Journal of Neurophysiology 76: 2547-2554.

Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19: 679-686.

Gil Z, Connors BW, Amitai Y (1999) Efficacy of thalamocortical and intracortical synaptic connections: Quanta, innervation, and reliability. Neuron 23: 385-397.

Gilbert CD, Wiesel TN (1992) Receptive field dynamics in adult primary visual cortex. Nature 356: 150-152.

Glazewski S, Barth AL, Wallace H, McKenna M, Silva A, Fox K (1999) Impaired experience-dependent plasticity in barrel cortex of mice lacking the alpha and delta isoforms of CREB. Cerebral Cortex 9: 249-256.

Glazewski S, Bejar R, Mayford M, Fox K (2001) The effect of autonomous alpha-CaMKII expression on sensory responses and experience-dependent plasticity in mouse barrel cortex. Neuropharmacology 41: 771-778.

Glazewski S, Chen CM, Silva A, Fox K (1996) Requirement for alpha-CaMKII in experience-dependent plasticity of the barrel cortex. Science 272: 421-423.

Glazewski S, Fox K (1996) Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. Journal of Neurophysiology 75: 1714-1729.

Glazewski S, Giese KP, Silva A, Fox K (2000) The role of alpha-CaMKII autophosphorylation in neocortical experience-dependent plasticity. Nature Neuroscience 3: 911-918.

Glazewski S, Herman C, McKenna M, Chapman PF, Fox K (1998a) Long-term potentiation in vivo in layers II/III of rat barrel cortex. Neuropharmacology 37: 581-592.

Glazewski S, McKenna M, Jacquin M, Fox K (1998b) Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex. European Journal of Neuroscience 10: 2107-2116.

Goldreich D, Kyriazi HT, Simons DJ (1999) Functional independence of layer IV barrels in rodent somatosensory cortex. Journal of Neurophysiology 82: 1311-1316.

Gottlieb JP, Keller A (1997) Intrinsic circuitry and physiological properties of pyramidal neurons in rat barrel cortex. Experimental Brain Research 115: 47-60.

Grenier F, Timofeev I, Steriade M (1998) Leading role of thalamic over cortical neurons during postinhibitory rebound excitation. Proceedings of the National Academy of Sciences of the United States of America 95: 13929-13934.

Grewer C, Watzke N, Wiessner M, Rauen T (2000) Glutamate translocation of the neuronal glutamate transporter EAAC1 occurs within milliseconds. Proceedings of the National Academy of Sciences of the United States of America 97: 9706-9711.

Hage I (2003) Early postnatal development of thalamocortical axons to barel cortex in NOR and barrelless mice: a morpholgical tracing study. University of Lausanne.

Harris EW, Ganong AH, Cotman CW (1984) Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Research 323: 132-137.

Harris KM (1999) Structure, development, and plasticity of dendritic spines. Current Opinion in Neurobiology 9: 343-348.

Harris RM, Woolsey TA (1981) Dendritic plasticity in mouse barrel cortex following postnatal vibrissa follicle damage. Journal of Comparative Neurology 196: 357-376.

Harris RM, Woolsey TA (1983) Computer-assisted analyses of barrel neuron axons and their putative synaptic contacts. Journal of Comparative Neurology 220: 63-79.

Hebb DO (1949) The organization of behaviour: A neuropsychological theory. New York: Wiley.

Hendry SH, Jones EG (1986) Reduction in number of immunostained GABAergic neurones in deprived-eye dominance columns of monkey area 17. Nature 320: 750-753.

Heynen AJ, Yoon BJ, Liu CH, Chung HJ, Huganir RL, Bear MF (2003) Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nature Neuroscience 6: 854-862.

Hickmott PW, Merzenich MM (2002) Local circuit properties underlying cortical reorganization. Journal of Neurophysiology 88: 1288-1301.

Higley MJ, Contreras D (2003) Nonlinear integration of sensory responses in the rat barrel cortex: an intracellular study in vivo. Journal of Neuroscience 23: 10190-10200.

Hoeflinger BF, Bennettclarke CA, Chiaia NL, Killackey HP, Rhoades RW (1995) Patterning of local intracortical projections within the vibrissae representation of rat primary somatosensory cortex. Journal of Comparative Neurology 354: 551-563.

Huang YH, Bergles DE (2004) Glutamate transporters bring competition to the synapse. Current Opinion in Neurobiology 14: 346-352.

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. Journal of Physiology 160: 106-154.

Huguenard JR (1996) Low-threshold calcium currents in central nervous system neurons. Annual Review of Physiology 58: 329-348.

Isaacson JS, Solis JM, Nicoll RA (1993) Local and diffuse synaptic actions of GABA in the hippocampus. Neuron 10: 165-175.

Ito M (1988) Response properties and topography of vibrissa-sensitive Vpm neurons in the rat. Journal of Neurophysiology 60: 1181-1197.

Izraeli R, Porter LL (1995) Vibrissal motor cortex in the rat: connections with the barrel field. Experimental Brain Research 104: 41-54.

Jeanmonod D, Rice FL, Van Der Loos H (1981) Mouse somatosensory cortex - Alterations in the barrelfield following receptor injury at different early postnatal ages. Neuroscience 6: 1503-1535.

Jensen KF, Killackey HP (1987) Terminal arbors of axons projecting to the somatosensory cortex of the adult-rat .1. the Normal Morphology of Specific Thalamocortical Afferents. Journal of Neuroscience 7: 3529-3543.

Jones EG (1993) GABAergic neurons and their role in cortical plasticity in primates. Cerebral Cortex 3: 361-372.

Kaas JH, Krubitzer LA, Chino YM, Langston AL, Polley EH, and Blair N (1990) Reorganization of retinotopic cortical maps in adult mammals after lesions of the retina. Science 248, 229-231.

Kaas JH, Merzenich MM, Killackey HP (1983) The reorganization of somatosensory cortex following peripheral-nerve damage in adult and developing mammals. Annual Review of Neuroscience 6: 325-356.

Kandel ER, schartz JH, Kessel TM (2000) Principles of neural science. Portland: Book News.

Katz LC, Crowley JC (2002) Development of cortical circuits: Lessons from ocular dominance columns. Nature Reviews Neuroscience 3: 34-42.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274: 1133-1138.

Keller A, Carlson GC (1999) Neonatal whisker clipping alters intracortical, but not thalamocortical projections, in rat barrel cortex. Journal of Comparative Neurology 412: 83-94.

Keller A, White EL (1987) Synaptic organization of GABAergic neurons in the mouse SmI cortex. Journal of Comparative Neurology 262: 1-12.

Keller A, White EL (1989) Triads - A Synaptic Network Component in the Cerebral-Cortex. Brain research 496: 105-112.

Keller A, White EL, Cipolloni PB (1985) The identification of thalamocortical axon terminals in barrels of mouse Sml cortex using immunohistochemistry of anterogradely transported lectin (Phaseolus-Vulgaris-Leucoagglutinin). Brain research 343: 159-165.

Killackey HP (1973) Anatomical evidence for cortical subdivisions based on vertically discrete thalamic projections from ventral posterior nucleus to cortical barrels in rat. Brain research 51: 326-331.

Killackey HP, Belford G, Ryugo R, Ryugo DK (1976) Anomalous organization of thalamocortical projections consequent to vibrissae removal in newborn rat and mouse. Brain research 104: 309-315.

Kim U, Ebner FF (1999) Barrels and septa: separate circuits in rat barrel field cortex. Journal of Comparative Neurology 408: 489-505.

Kleinfeld D, Delaney KR (1996) Distributed representation of vibrissa movement in the upper layers of somatosensory cortex revealed with voltage-sensitive dyes. Journal of Comparative Neurology 375: 89-108.

Knott GW, Quairiaux C, Genoud C, Welker E (2002) Formation of dendritic spines with GABAergic synapses by whisker stimulation in induced adult mice. Neuron 34: 265-273.

Kohara K, Kitamura A, Adachi N, Nishida M, Itami C, Nakamura S, Tsumoto T (2003) Inhibitory but not excitatory cortical neurons require presynaptic brain-derived neurotrophic factor for dendritic development, as revealed by chimera cell culture. Journal of Neuroscience 23: 6123-6131.

Koralek KA, Jensen KF, Killackey HP (1988) Evidence for 2 complementary patterns of thalamic input to the rat somatosensory cortex. Brain research 463: 346-351.

Kossut M (1998) Experience-dependent changes in function and anatomy of adult barrel cortex. Experimental Brain Research 123: 110-116.

Kossut M, Hand PJ, Greenberg J, Hand CL (1988) Single vibrissal cortical column in SI cortex of rat and its alterations in neonatal and adult vibrissa-deafferented animals: a quantitative 2DG study. Journal of Neurophysiology 60: 829-852.

Krupa DJ, Ghazanfar AA, Nicolelis MA (1999) Immediate thalamic sensory plasticity depends on corticothalamic feedback. Proceedings of the National Academy of Sciences of the United States of America 96: 8200-8205.

Kyriazi H, Carvell GE, Brumberg JC, Simons DJ (1998) Laminar differences in bicuculline methiodide's effects on cortical neurons in the rat whisker barrel system. Somatosensory and Motor Research 15: 146-156.

Kyriazi HT, Carvell GE, Brumberg JC, Simons DJ (1996a) Effects of baclofen and phaclofen on receptive field properties of rat whisker barrel neurons. Brain Research 712: 325-328.

Kyriazi HT, Carvell GE, Brumberg JC, Simons DJ (1996b) Quantitative effects of GABA and bicuculline methiodide on receptive field properties of neurons in real and simulated whisker barrels. Journal of Neurophysiology 75: 547-560.

Kyriazi HT, Carvell GE, Simons DJ (1994) OFF response transformations in the whisker/barrel system. Journal of Neurophysiology 72: 392-401.

Kyriazi HT, Simons DJ (1993) Thalamocortical response transformations in simulated whisker barrels. Journal of Neuroscience 13: 1601-1615.

Laaris N, Carlson GC, Keller A (2000) Thalamic-evoked synaptic interactions in barrel cortex revealed by optical imaging. Journal of Neuroscience 20: 1529-1537.

Laaris N, Keller A (2002) Functional independence of layer IV barrels. Journal of Neurophysiology 87: 1028-1034.

Lampl I, Reichova I, Ferster D (1999) Synchronous membrane potential fluctuations in neurons of the cat visual cortex. Neuron 22: 361-374.

Land PW, Buffer SA, Yaskosky JD (1995) Barreloids in adult-rat thalamus - 3-dimensional architecture and relationship to somatosensory cortical barrels. Journal of Comparative Neurology 355: 573-588.

Lebedev MA, Mirabella G, Erchova I, Diamond ME (2000) Experience-dependent plasticity of rat barrel cortex: Redistribution of activity across barrel-columns. Cerebral Cortex 10: 23-31.

Lee KJ, Woolsey TA (1975) Proportional relationship between peripheral innervation density and cortical neuron number in somatosensory system of mouse. Brain research 99: 349-353.

Lee SM, Weisskopf MG, Ebner FF (1991) Horizontal long-term potentiation of responses in rat somatosensory cortex. Brain Research 544: 303-310.

Lehre KP, Danbolt NC (1998a) Determination of the number of glial glutamate transporter molecules and astroglial surface area in subregions of the rat hippocampus and cerebellum. European Journal of Neuroscience 10: 351.

Lehre KP, Danbolt NC (1998b) The number of glutamate transporter subtype molecules at glutamatergic synapses: Chemical and stereological quantification in young adult rat brain. Journal of Neuroscience 18: 8751-8757.

Lehre KP, Levy LM, Chaudhry FA, Stormmathisen J, Ottersen OP, Danbolt NC (1993) Localization of glutamate transporters in brain by site-directed immunocytochemistry. Journal of Neurochemistry 61: S251.

Lehre KP, Levy LM, Ottersen OP, Stormmathisen J, Danbolt NC (1995) Differential Expression of 2 glial glutamate transporters in the rat-brain - Quantitative and immunocytochemical observations. Journal of Neuroscience 15: 1835-1853.

Lendvai B, Stern EA, Chen B, Svoboda K (2000) Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature 404: 876-881.

Leung L-WS (1990) Field potentials in the central nervous system: recording, analysis, and modeling. In: Neurophysiological Techniques, II Applications to Neural Systems: 277-312.

Levay S, Stryker MP, Shatz CJ (1978) Ocular dominance columns and their development in layer 4 of cats visual-cortex - Quantitative study. Journal of Comparative Neurology 179: 223-244.

Levay S, Wiesel TN, Hubel DH (1980) The development of ocular dominance columns in normal and visually deprived monkeys. Journal of Comparative Neurology 191: 1-51.

Levenson J, Weeber E, Selcher JC, Kategaya LS, Sweatt JD, Eskin A (2002) Long-term potentiation and contextual fear conditioning increase neuronal glutamate uptake. Nature Neuroscience 5: 155-161.

Levy LM, Lehre KP, Rolstad B, Danbolt NC (1993a) A monoclonal-antibody raised against an [Na+ + K+] coupled L-glutamate transporter purified from rat-brain confirms glial-cell localization. Federation of European Biochemical Societies Letters 317: 79-84.

Levy LM, Lehre KP, Walaas I, Stormmathisen J, Danbolt NC (1993b) Down-regulation of a glial glutamate transporter in striatum after destruction of the glutamatergic corticostriatal projection. Journal of Neurochemistry 61: S208.

Levy LM, Lehre KP, Walaas SI, Stormmathisen J, Danbolt NC (1995) Down-regulation of glial glutamate transporters after glutamatergic denervation in the rat-brain. European Journal of Neuroscience 7: 2036-2041.

Lorente de Nò R (1922) Contribution al conocimiento del nervo trigemino. In: Libro en Honor. edited by Ramon y Cajal S. Madrid, Spain: Jaminez y Molina, 1922, p. 13-29..

Lowel S, Singer W (1992) Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. Science 255: 209-212.

Lu SM, Lin RCS (1993) Thalamic afferents of the rat barrel cortex - A light-microscopic and electron-microscopic study using Phaseolus-Vulgaris Leukoagglutinin as an anterograde tracer. Somatosensory and Motor Research 10: 1-16.

Lubke J, Egger V, Sakmann B, Feldmeyer D (2000) Columnar organization of dendrites and axons of single and synaptically coupled excitatory spiny neurons in layer 4 of the rat barrel cortex. Journal of Neuroscience 20: 5300-5311.

Lübke J, Egger V, Sakmann B and Feldmeyer D (2000) Columnar organization of dendrites and axons of single and synaptically coupled excitatory spiny neurons in layer 4 of the rat barrel cortex. The journal of neuroscience 20: 5300-5311.

Lubke J, Roth A, Feldmeyer D, Sakmann B (2003) Morphometric analysis of the columnar innervation domain of neurons connecting layer 4 and layer 2/3 of juvenile rat barrel cortex. Cerebral Cortex 13: 1051-1063.

Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression - Postsynaptic correlate of long-term potentiation. Nature 266: 737-739.

Ma PM (1991) The barrelettes - Architectonic vibrissal representations in the brain-stem trigeminal complex of the mouse .1. Normal structural organization. Journal of Comparative Neurology 309: 161-199.

Manns ID, Sakmann B, Brecht M (2004) Sub- and suprathreshold receptive field properties of pyramidal neurones in layers 5A and 5B of rat somatosensory barrel cortex. Journal of Physiology 556: 601-622.

Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275: 213-215.

Martin SJ, Grimwood PD, Morris RGM (2000) Synaptic plasticity and memory: An evaluation of the hypothesis. Annual Review of Neuroscience 23: 649-711.

Marty A, Llano I (1995) Modulation of inhibitory synapses in the mammalian brain. Current Opinion in Neurobiology 5: 335-341.

Marty S, Berzaghi MD, Berninger B (1997) Neurotrophins and activity-dependent plasticity of cortical interneurons. Trends in Neurosciences 20: 198-202.

Marty S, Wehrle R, Sotelo C (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. Journal of Neuroscience 20: 8087-8095.

Mccasland JS, Bernardo KL, Probst KL, Woolsey TA (1992) Cortical local circuit axons do not mature after early deafferentation. Proceedings of the National Academy of Sciences of the United States of America 89: 1832-1836.

Mccormick DA, Connors BW, Lighthall JW, Prince DA (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. Journal of Neurophysiology 54: 782-806.

Melzer P, Van Der Loos H, Dörfl J, Welker E, Robert P, Emery D and Berrini JC (1985) A magnetic device to stimulate selected whiskers of freely moving or restrained small rodents : its application in a deoxyglucose study. Brain Research 348, 229-240.

Mennerick S, Zorumski CF (1994) Glial contributions to excitatory neurotransmission in cultured hippocampal cells. Nature 368: 59-62.

Merzenich MM, Kaas JH, Wall J, Nelson RJ, Sur M, Felleman D (1983) Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience 8: 33-55.

Merzenich MM, Nelson RJ, Stryker MP, Cynader MS, Schoppmann A, Zook JM (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. Journal of Comparative Neurology 224: 591-605.

Micheva KD, Beaulieu C (1995) An anatomical substrate for experience-dependent plasticity of the rat barrel field cortex. Proceedings of the National Academy of Sciences of the United States of America 92: 11834-11838.

Micheva KD, Beaulieu C (1996) Quantitative aspects of synaptogenesis in the rat barrel field cortex with special reference to GABA circuitry. Journal of Comparative Neurology 373: 340-354.

Miller KD, Pinto DJ, Simons DJ (2001) Processing in layer 4 of the neocortical circuit: new insights from visual and somatosensory cortex. Current Opinion in Neurobiology 11: 488-497.

Mirabella G, Battiston S, Diamond ME (2001) Integration of multiple-whisker inputs in rat somatosensory cortex. Cerebral Cortex 11: 164-170.

Mitzdorf U (1985) Current source-density method and application in cat cerebral-cortex - investigation of evoked-potentials and EEG phenomena. Physiological Reviews 65: 37-100.

Moore CI (2004) Frequency-dependent processing in the vibrissa sensory system. Journal of Neurophysiology 91: 2390-2399.

Moore CI, Nelson SB (1998) Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. Journal of Neurophysiology 80: 2882-2892.

Moore CI, Nelson SB, Sur M (1999) Dynamics of neuronal processing in rat somatosensory cortex. Trends in Neurosciences 22: 513-520.

Mountcastle VB (1957) Modality and topographic properties of single neurons of cats Somatic sensory cortex. Journal of Neurophysiology 20: 408-434.

Nicholson C, Freeman JA (1975) Theory of current source-density analysis and determination of conductivity tensor for anuran cerebellum. Journal of Neurophysiology 38: 356-368.

Nieuwenhuys R, Voogd J, Van Huijzen C (1981) The human central nervous system, Springer-Verlag, 1978. New york: Springer-Verlag.

Oliet SHR, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 292: 923-926.

Overstreet LS, Kinney GA, Liu YB, Billups D, Slater NT (1999) Glutamate transporters contribute to the time course of synaptic transmission in cerebellar granule cells. Journal of Neuroscience 19: 9663-9673.

Perego C, Vanoni C, Bossi M, Massari S, Basudev H, Longhi R, Pietrini G (2000) The GLT-1 and GLAST glutamate transporters are expressed on morphologically distinct astrocytes and regulated by neuronal activity in primary hippocampal cocultures. Journal of Neurochemistry 75: 1076-1084.

Petersen CCH (2002) Short-term dynamics of synaptic transmission within the excitatory neuronal network of rat layer 4 barrel cortex. Journal of Neurophysiology 87: 2904-2914.

Petersen CCH, Grinvald A, Sakmann B (2003a) Spatiotemporal dynamics of sensory responses in layer 2/3 of rat barrel cortex measured in vivo by voltage-sensitive dye imaging combined with whole-cell voltage recordings and neuron reconstructions. Journal of Neuroscience 23: 1298-1309.

Petersen CCH, Hahn TTG, Mehta M, Grinvald A, Sakmann B (2003b) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proceedings of the National Academy of Sciences of the United States of America 100: 13638-13643.

Petersen CCH, Sakmann B (2000) The excitatory neuronal network of rat layer 4 barrel cortex. Journal of Neuroscience 20: 7579-7586.

Petersen CCH, Sakmann B (2001) Functionally independent columns of rat somatosensory barrel cortex revealed with voltage-sensitive dye imaging. Journal of Neuroscience 21: 8435-8446.

Petersen RS, Diamond ME (2000) Spatial-temporal distribution of whisker-evoked activity in rat somatosensory cortex and the coding of stimulus location. Journal of Neuroscience 20: 6135-6143.

Peterson BE, Goldreich D, Merzenich MM (1998) Optical imaging and electrophysiology of rat barrel cortex. I. Responses to small single-vibrissa deflections. Cerebral Cortex 8: 173-183.

Pinto DJ, Brumberg JC, Simons DJ (2000) Circuit dynamics and coding strategies in rodent somatosensory cortex. Journal of Neurophysiology 83: 1158-1166.

Poitry-Yamate CL, Vutskits L, Rauen T (2002) Neuronal-induced and glutamate-dependent activation of glial glutamate transporter function. Journal of Neurochemistry 82: 987-997.

Polley DB, Chen-Bee CH and Frostig RD (1999) Two directions of plasticity in the sensory-deprived adult cortex. Neuron 24, 623-637.

Pons TP, Garraghty PE, Ommaya AK, Kaas JH, Taub E, Mishkin M (1991) Massive cortical reorganization after sensory deafferentation in adult macaques. Science 252: 1857-1860.

Porter JT, Johnson CK, Agmon A (2001) Diverse types of interneurons generate thalamus-evoked feedforward inhibition in the mouse barrel cortex. Journal of Neuroscience 21: 2699-2710.

Porter JT, Nieves D (2004) Presynaptic GABAB receptors modulate thalamic excitation of inhibitory and excitatory neurons in the mouse barrel cortex. Journal of Neurophysiology 92: 2762-2770.

Quairiaux C, Genoud C, Steiner P, Hirling A, Welker E, and Knott GW (2005) Astroglial plasticity in adult mice somatosensory cortex following in vivo sensory stimulation. In preparation. 2005.

Rauen T, Wiessner M, Watzke N, Grewer C (2000) Rapid kinetics of the neuronal glutamate transporter EAAC1 and its impact on synaptic signalling. European Journal of Neuroscience 12: 464.

Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, Dinse HR (1992) Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. Journal of Neurophysiology 67: 1031-1056.

Rema V, Armstrong-James M and Ebner FF (1998) Experience-dependent plasticity of adult rat S1 cortex requires local NMDA receptor activation. The journal of neuroscience 18(23)[December 1], 10196-10206.

Rema V, Armstrong-James M, Ebner FF (2003) Experience-dependent plasticity is impaired in adult rat barrel cortex after whiskers are unused in early postnatal life. Journal of Neuroscience 23: 358-366.

Rice FL, Anders D (1977) Small guillotine to prepare brains for sectioning in defined, odd planes. Neuroscience Letters 6: 157-163.

Rice FL, Van Der Loos H (1977) Development of barrels and barrel field in somatosensory cortex of mouse. Journal of Comparative Neurology 171: 545-560.

Robertson D, Irvine DR (1989) Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. Journal of Comparative Neurology 282: 456-471.

Rocamora N, Welker E, Pascual M, Soriano E (1996) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. Journal of Neuroscience 16: 4411-4419.

Rossi DJ, Hamann M (1998) Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha(6) subunit GABA(A) receptors and glomerular geometry. Neuron 20: 783-795.

Rothstein JD, DykesHoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang YF, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16: 675-686.

Rothstein JD, Martin L, Levey AI, DykesHoberg M, Jin L, Wu D, Nash N, Kuncl RW (1994) Localization of neuronal and glial glutamate transporters. Neuron 13: 713-725.

Schlaggar BL, Fox K, Oleary DDM (1993) Postsynaptic control of plasticity in developing somatosensory cortex. Nature 364: 623-626.

Schubert D, Kotter R, Zilles K, Luhmann HJ, Staiger JF (2003) Cell type-specific circuits of cortical layer IV spiny neurons. Journal of Neuroscience 23: 2961-2970.

Schubert D, Staiger JF, Cho N, Kotter R, Zilles K, Luhmann HJ (2001) Layer-specific intracolumnar and transcolumnar functional connectivity of layer V pyramidal cells in rat barrel cortex. Journal of Neuroscience 21: 3580-3592.

Seil FJ, Drake-Baumann R (2000) TrkB receptor ligands promote activity-dependent inhibitory synaptogenesis. Journal of Neuroscience 20: 5367-5373.

Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nature Neuroscience 6: 484-490.

Shepherd GMG, Pologruto TA, Svoboda K (2003) Circuit analysis of experience-dependent plasticity in the developing rat barrel cortex. Neuron 38: 277-289.

Shibata T, Yamada K, Watanabe M, Ikenaka K, Wada K, Tanaka K, Inoue Y (1997) Glutamate transporter GLAST is expressed in the radial glia-astrocyte lineage of developing mouse spinal cord. Journal of Neuroscience 17: 9212-9219.

Shieh PB, Hu SC, Bobb K, Timmusk T, Ghosh A (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. Neuron 20: 727-740.

Shoykhet M, Doherty D, Simons DJ (2000) Coding of deflection velocity and amplitude by whisker primary afferent neurons: implications for higher level processing. Somatosensory and Motor Research 17: 171-180.

Silver RA, Lubke J, Sakmann B, Feldmeyer D (2003) High-probability uniquantal transmission at excitatory synapses in barrel cortex. Science 302: 1981-1984.

Simons DJ (1978) Response properties of vibrissa units in rat SI somatosensory neocortex. Journal of Neurophysiology 41: 798-820.

Simons DJ (1985) Temporal and spatial integration in the rat SI vibrissa cortex. Journal of Neurophysiology 54: 615-635.

Simons DJ, Carvell GE (1989) Thalamocortical response transformation in the rat vibrissa barrel system. Journal of Neurophysiology 61: 311-330.

Simons DJ, Carvell GE, Hershey AE, Bryant DP (1992) Responses of barrel cortex neurons in awake rats and effects of urethane anesthesia. Experimental Brain Research 91: 259-272.

Simons DJ, Land PW (1987) Early experience of tactile stimulation influences organization of somatic sensory cortex. Nature 326: 694-697.

Simons DJ, Woolsey TA (1984) Morphology of golgi-cox-impregnated barrel neurons in rat SmI cortex. Journal of Comparative Neurology 230: 119-132.

Siucinska E, Kossut M (1996) Short-lasting classical conditioning induces reversible changes of representational maps of vibrissae in mouse SI cortex--a 2DG study. Cerebral Cortex 6: 506-513.

Sosnik R, Haidarliu S, Ahissar E (2001) Temporal frequency of whisker movement. I. Representations in brain stem and thalamus. Journal of Neurophysiology 86: 339-353.

Stafstrom CE, Schwindt PC, Flatman JA, Crill WE (1984) Properties of subthreshold response and action-potential recorded in layer-V neurons from cat sensorimotor cortex in vitro. Journal of Neurophysiology 52: 244-263.

Staiger JF, Flagmeyer I, Schubert D, Zilles K, Kotter R, Luhmann HJ (2004) Functional diversity of layer IV spiny neurons in rat somatosensory cortex: Quantitative morphology of electrophysiologically characterized and biocytin labeled cells. Cerebral Cortex 14: 690-701.

Staiger JF, Kotter R, Zilles K, Luhmann HJ (2000) Laminar characteristics of functional connectivity in rat barrel cortex revealed by stimulation with caged-glutamate. Neuroscience Research 37: 49-58.

Staiger JF, Zilles K, Freund TF (1996a) Distribution of GABAergic elements postsynaptic to ventroposteromedial thalamic projections in layer IV of rat barrel cortex. European Journal of Neuroscience 8: 2273-2285.

Staiger JF, Zilles K, Freund TF (1996b) Recurrent axon collaterals of corticothalamic projection neurons in rat primary somatosensory cortex contribute to excitatory and inhibitory feedback-loops. Anatomy and Embryology 194: 533-543.

Stent GS (1973) Physiological mechanism for Hebbs postulate of learning. Proceedings of the National Academy of Sciences of the United States of America 70: 997-1001.

Steriade M, Nunez A, Amzica F (1993) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. Journal of Neuroscience 13: 3252-3265.

Steriade M, Timofeev I (1997) Short-term plasticity during intrathalamic augmenting responses in decorticated cats. Journal of Neuroscience 17: 3778-3795.

Stern EA, Maravall M, Svoboda K (2001) Rapid development and plasticity of layer 2/3 maps in rat barrel cortex in vivo. Neuron 31: 305-315.

Swadlow HA, Gusev AG (2000) The influence of single VB thalamocortical impulses on barrel columns of rabbit somatosensory cortex. Journal of Neurophysiology 83: 2802-2813.

Szabadics J, Lorincz A, Tamas G (2001) Beta and gamma frequency synchronization by dendritic gabaergic synapses and gap junctions in a network of cortical interneurons. Journal of Neuroscience 21: 5824-5831.

Takahashi M, Attwell D (1995) Postsynaptic glutamate uptake helps shape the EPSC at climbing fiber to Purkinje-cell synapses in rat cerebellar slices. Journal of Physiology-London 487P: 49-50.

Takahashi T, Svoboda K, Malinow R (2003) Experience strengthening transmission by driving AMPA receptors into synapses. Science 299: 1585-1588.

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Hori S, Takimoto M, Wada K (1997) Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276: 1699-1702.

Torp R, Danbolt NC, Babaie E, Bjoras M, Seeberg E, Stormmathisen J, Ottersen OP (1994) Differential expression of 2 glial glutamate transporters in the rat-brain - An in-situ hybridization study. European Journal of Neuroscience 6: 936-942.

Trachtenberg JT, Chen BE, Knott GW, Feng GP, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420: 788-794.

Ullensvang K, Lehre KP, Stormmathisen J, Danbolt NC (1997) Differential developmental expression of the two rat brain glutamate transporter proteins GLAST and GLT. European Journal of Neuroscience 9: 1646-1655.

Urban J, Kossut M, Hess G (2002) Long-term depression and long-term potentiation in horizontal connections of the barrel cortex. European Journal of Neuroscience 16: 1772-1776.

Valverde F (1986) Intrinsic neocortical organization - Some comparative aspects. Neuroscience 18: 1-23.

Van Der Loos H (1976) Barreloids in mouse somatosensory thalamus. Neuroscience Letters 2: 1-6.

Van Der Loos H, Welker E, Dorfl J, Rumo G (1986) Selective breeding for variations in patterns of mystacial vibrissae of mice - Bilaterally symmetrical strains derived from ICR stock. Journal of Heredity 77: 66-82.

Van Der Loos H and Woolsey TA (1973) Somatosensory cortex: structural alterations following early injury to sense organs. Science 179, 395-398.

Vincent SB (1912) The function of the vibrissae in the behaviour of the white rat. Behavioural monographs 1, 1-81.

Wallace H, Fox K (1999) The effect of vibrissa deprivation pattern on the form of plasticity induced in rat barrel cortex. Somatosensory and Motor Research 16: 122-138.

Wallace H, Glazewski S, Liming K, Fox K (2001) The role of cortical activity in experience-dependent potentiation and depression of sensory responses in rat barrel cortex. Journal of Neuroscience 21: 3881-3894.

Wang X, Merzenich MM, Sameshima K, Jenkins WM (1995) Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. Nature 378: 71-75.

Watanabe T, Morimoto K, Hirao T, Suwaki H, Watase K, Tanaka K (1999) Amygdala-kindled and pentylenetetrazole-induced seizures in glutamate transporter GLAST-deficient mice. Brain Research 845: 92-96.

Weinberger NM (1993) Learning-induced changes of auditory receptive fields. Current Opinion in Neurobiology 3: 570-577.

Welker C (1976) Receptive-fields of barrels in somatosensory neocortex of rat. Journal of Comparative Neurology 166: 173-189.

Welker E, Armstrong-James M, Van Der Loos H, Kraftsik R (1993) The Mode of activation of a barrel column - Response properties of single units in the somatosensory cortex of the mouse upon whisker deflection. European Journal of Neuroscience 5: 691-712.

Welker E, Armstrongjames M, Bronchti G, Ourednik W, Gheorghita-Baechler F, Dubois R, Guernsey DL, Van Der Loos H, Neumann PE (1996) Altered sensory processing in the somatosensory cortex of the mouse mutant barrelless. Science 271: 1864-1867.

Welker E, Hoogland PV, Van Der Loos H (1988) Organization of feedback and feedforward projections of the barrel cortex: a PHA-L study in the mouse. Experimental Brain Research 73: 411-435.

Welker E, Rao SB, Dörfl J, Melzer P and Van Der Loos H (1992) Plasticity in the barrel cortex of the adult mouse:effects of chronic stimulation upon deoxyglucose uptake in the behaving animal. The journal of neuroscience 12: 153-170.

Welker E, Soriano E, Dörfl J and Van Der Loos, H (1989a) Plasticity in the barrel cortex of the adult mouse: transient increase of GAD-immunoreactivity following sensory stimulation. Experimental Brain Research 78, 659-664.

Welker E, Soriano E and Van Der Loos H (1989b) Plasticity in the barrel cortex of the adult mouse: effects of peripheral deprivation on GAD-immunoreactivity. Experimental Brain Research 73, 441-452.

Welker E, Van Der Loos H (1986) Is areal extent in sensory cerebral-cortex determined by peripheral innervation density. Experimental Brain Research 63: 650-654.

Welker E, Van Der Loos H, Dörfl J, Soriano E (1990) The possible role of gabaergic innervation in plasticity of adult cerebral cortex. In: The neocortex ontogeny and phylogeny pp 237-243. New-York: Finlay,B.L.; Innocenti,G.; Scheich,H; Plenum.

Welker WI (1964) Analysis of sniffing of the albino rat. Behaviour 22, 223-244.

White EL (1978) Identified neurons in mouse Sml cortex which are postsynaptic to thalamocortical axon terminals - Combined golgi electron microscopic and degeneration study. Journal of Comparative Neurology 181: 627-661.

White EL (1989) Cortical circuits: synaptic organization of the cerebral cortex. Boston.

White EL, Keller A, Cipolloni PB (1985) Quantification of thalamocortical axon terminals using immunohistochemistry of anterogradely transported Lectin. Anatomical Record 211: A213-A214.

White EL, Rock MP (1980a) 3-Dimensional aspects and synaptic relationships of a Golgi-impregnated spiny stellate cell reconstructed from serial thin-sections. Journal of Neurocytology 9: 615-30.

White EL, Rock MP (1980b) Three-dimensional aspects and synaptic relationships of a Golgi-impregnated spiny stellate cell reconstructed from serial thin sections. Journal of Neurocytology 9: 615-636.

Wiesel TN, Hubel DH (1963a) Effects of visual deprivation on morphology and physiology of cells in cats lateral geniculate body. Journal of Neurophysiology 26: 978-987.

Wiesel TN, Hubel DH (1963b) Single-cell responses in striate cortex of kittens deprived of vision in 1 eye. Journal of Neurophysiology 26: 1003-1012.

Wiesel TN, Hubel DH (1965) Comparison of effects of unilateral and bilateral eye closure on cortical unit responses in kittens. Journal of Neurophysiology 28: 1029-1040.

Wilent WB, Contreras D (2004) Synaptic responses to whisker deflections in rat barrel cortex as a function of cortical layer and stimulus intensity. Journal of Neuroscience 24: 3985-3998.

Wirth C, Luscher HR (2004) Spatiotemporal evolution of excitation and inhibition in the rat barrel cortex investigated with multielectrode arrays. Journal of Neurophysiology 91: 1635-1647.

Woodin MA, Ganguly K, Poo MM (2003) Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl- transporter activity. Neuron 39: 807-820.

Woolsey TA, Van Der Loos H (1970) Structural organization of layer-IV in somatosensory region (SI) of mouse cerebral cortex . Description of A Cortical Field Composed of Discrete Cytoarchitectonic Units. Brain research 17: 205-215.

Woolsey TA, Wann JR (1976) Areal changes in mouse cortical barrels following vibrissal damage at different postnatal ages. Journal of Comparative Neurology 170: 53-66.

Yamada K, Watanabe M, Shibata T, Nagashima M, Tanaka K, Inoue Y (1998) Glutamate transporter GLT-1 is transiently localized on growing axons of the mouse spinal cord before establishing astrocytic expression. Journal of Neuroscience 18: 5706-5713.

Zhang ZW, Deschenes M (1997) Intracortical axonal projections of lamina VI cells of the primary somatosensory cortex in the rat: A single-cell labeling study. Journal of Neuroscience 17: 6365-6379.

Zhang ZW, Deschenes M (1998) Projections to layer VI of the posteromedial barrel field in the rat: a reappraisal of the role of corticothalamic pathways. Cerebral Cortex 8: 428-436.

Zhu JJ, Connors BW (1999) Intrinsic firing patterns and whisker-evoked synaptic responses of neurons in the rat barrel cortex. Journal of Neurophysiology 81: 1171-1183.

Zhu YH, Stornetta RL, Zhu JJ (2004) Chandelier cells control excessive cortical excitation: Characteristics of whisker-evoked synaptic responses of layer 2/3 nonpyramidal and pyramidal neurons. Journal of Neuroscience 24: 5101-5108.